Rotor-Gene
Software Manual

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1 Introduction

1.1 Important: Read Before Running The Rotor-Gene

Before running the Rotor-Gene you should pay attention to the following:

1) Always run the unit with 36 (32) or 72 tubes in the rotor. If you do not have this many samples load empty tubes into the rotor. This ensures the thermal load in the chamber is identical for every run. If 0.1ml tubes are used it is sufficient to place empty tubes without lids into the rotor.

Do not use caps on the blank tubes. If caps are loaded on blank tubes and run multiple times there is a risk that caps may come off the tube causing damage to the rotor.

72-well Locking Ring: To ensure that tubes sit firmly in the 72 well rotor, a 72 well locking ring is available. After loading the tubes, the rotor can simply be put over the rotor.

2) Ensure the software is not operating in the Virtual Machine Mode. Go to the **File menu** and select **Setup...** Check that the Virtual Machine box is not ticked.

If **Setup...** cannot be selected then it has been disabled and your distributor should have preset the software.

3) Before running a program ensure the Rotor-Gene shows **READY** on the display. If **READY** is not shown the machine will not set the Gains for the channels and no data will be collected.

1.1.1 Rotor-Gene Keyboard (Rotor-Gene 2000 Only)

The keyboard can be used to set the unit to a temperature and hold. In addition, the Rotor-Gene keyboard display shows the lid status, either Open or Closed.

Pressing the **Next key** advances the window forward to the next display and shows Lid Status then Set Temperature.

Use the **Up** and **Down** keys to change the set temperature, once set press **Start**.

The system then will go through a Start sequence that turns on the rotor, then the chamber fan and then the heater.

The unit will heat or cool and hold the set temperature. This is useful if incubation at a particular temperature is required before thermal cycling samples.

The keyboard can be used to set a manual temperature but is not used in regular operation.

1.1.2 Rotor-Gene Startup

The Rotor-Gene is setup using the following procedure:

1. Turn the desktop PC on and wait for Windows to boot up.
2. Turn on the Rotor-Gene on the left-hand side of the unit wait for the ready message to be displayed on the LCD.

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3. Double click on the Rotor-Gene icon.
4. The Rotor-Gene is now ready for use.

Note for Rotor-Gene 2000 users: If the Rotor-Gene is turned ON without having Rotor-Gene software loaded and running on the PC, the instrument will emit a buzzing sound. By running the Rotor-Gene software the buzzing will stop.

1.1.3 Software Version

Software development for the Rotor-Gene system is ongoing. To check on your version number click on **Help** then **About Rotor-Gene...** The latest software version is available for download at our web site.

rotor-gene
Version 4.7 (Build 1), 11/6/2002
Machine Serial No : 123456 Offset Coefficient : 0
Corbett Research
1/14 Hilly St
Mortlake NSW 2137
Australia
Ph : +612 9736 1320
Email: support@corbettresearch.com
Innovative instrumentation
for life sciences
Warning: This computer program is protected by copyright law and international treaties. Unauthorized reproduction of this program, or any portion of it, may result in severe civil and criminal penalties, and will be prosecuted to the maximum extent possible under the law.
OK
System Info...
Help

This screen displays general information about the software, and specifically includes the version of the software, serial number of the machine, as well as the date it was last updated.

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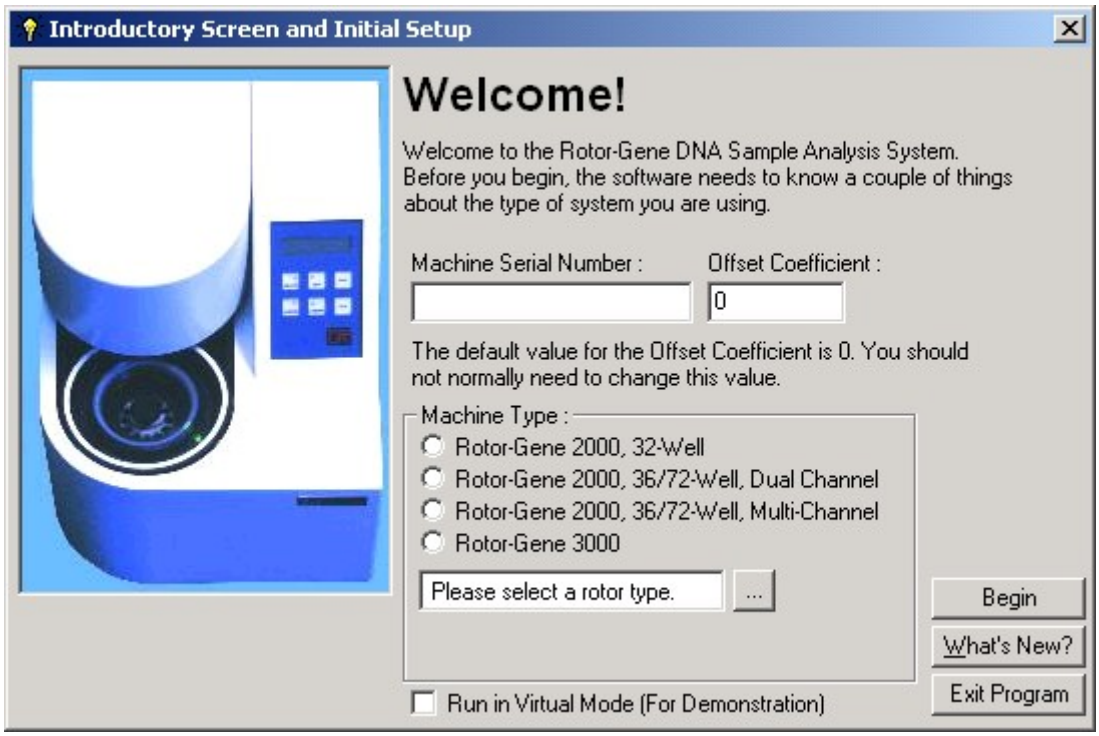
1.1.4 Welcome Screen

This screen appears when a new version of the Rotor-Gene software has been installed and the Rotor-Gene icon is double clicked for the first time after the installation.


Introductory Screen and Initial Setup
Welcome!
Welcome to the Rotor-Gene DNA Sample Analysis System.
Before you begin, the software needs to know a couple of things about the type of system you are using.
Machine Serial Number :
Offset Coefficient :
0
The default value for the Offset Coefficient is 0. You should not normally need to change this value.
Machine Type :
Rotor-Gene 2000, 32-Well
Rotor-Gene 2000, 36/72-Well, Dual Channel
Rotor-Gene 2000, 36/72-Well, Multi-Channel
Rotor-Gene 3000
Please select a rotor type.
...
Begin
What's New?
Exit Program
Run in Virtual Mode (For Demonstration)

Machine Serial Number: Type in the serial number (six digits), which is located at the back of the Rotor-Gene.

Offset Coefficient: Type in the Offset coefficient, which can be found next to the serial number of the machine. If no number can be found, the value 0 should be entered.

Machine Type: Chose your type of machine.

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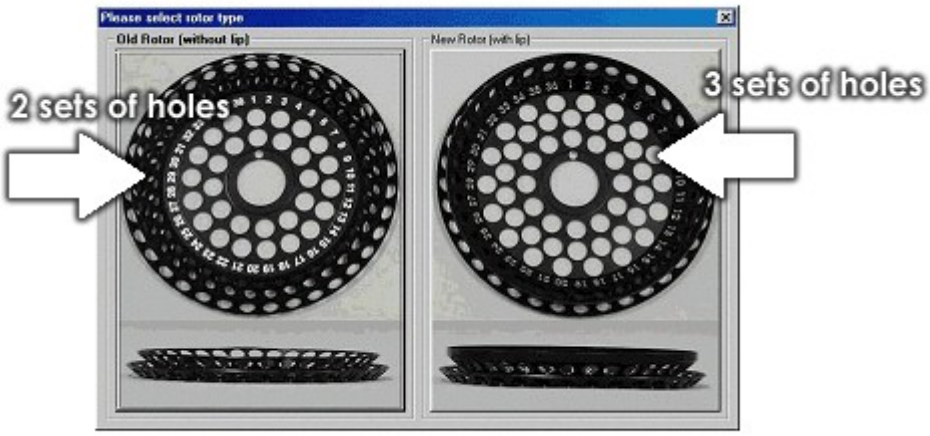
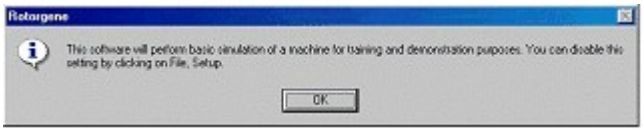
Choose type of 36 well Rotor:



Run in Virtual Mode (for demonstration): Ticking the box allows installing the Rotor-Gene software on a computer without Rotor-Gene attached. The software is fully functional and can even simulate runs.

NOTE: If this box is ticked with a Rotor-Gene connected to your computer, a message will appear before you start your run: "you about to run in virtual mode". To be able to perform a "real" run, the setup (see setup) has to be changed.

Begin: When all parameters are set, press Begin. A window will come up initializing machine. Wait until the machine is initialized, which might take a few seconds. If virtual mode was chosen the following screen appears:


Rotorgene
This software will perform basic simulation of a machine for training and demonstration purposes. You can disable this setting by clicking on File, Setup.
OK

If the box stays unticked the machine is initialized and opens up the Rotor-Gene software automatically.

What's new: This topic explains the new features in this version of Rotor-Gene and answers commonly asked questions about the location of existing features that have been moved. These features incorporate design changes and requests by users.

Exit Program: Exits program.

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2 Rotor-Gene Description

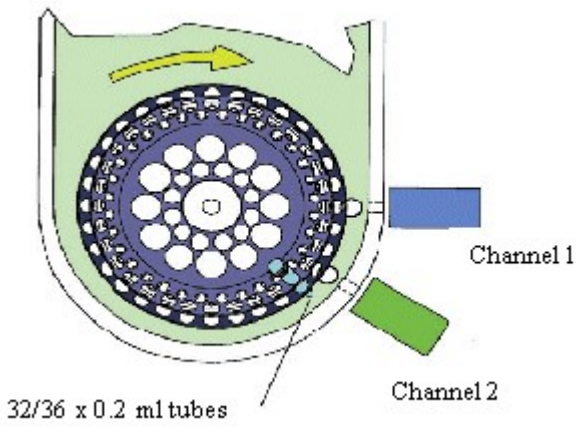
2.1 32-Well and Dual-Channel Rotor-Gene

The Rotor-Gene is a real-time thermal cycling system used for DNA amplification and hybridization. The unit holds 36 x 0.2ml standard micro-fuge tubes (32 x 0.2), which are loaded into a 36-position rotor (32). With a Dual-Channel machine, a 72-well rotor is also supplied that can run special strip tubes that are 0.1ml in strips of four. During the run the rotor spins at approximately 500 rpm as the tubes are thermally cycled in a low-mass air oven.

NOTE: Always run a full rotor of tubes, for both the 72-well and 36-well rotors. If the number of samples is small fill the remaining positions with empty tubes. This ensures the thermal load of plastic tubes is the same from run to run.

There are two detection modules in the Rotor-Gene, Channel (1) is setup to detect 510nm and Channel (2) is setup to detect 555nm. This allows for multiplexed samples to be run.

As the sample tubes spin past the detector modules, a high powered LED strobes the sample and a photomultiplier (PMP) collects the fluorescent energy. See the figure below.



This data is sent to a Desktop PC that averages the energy of each sample over a number of revolutions. This data is then displayed in real-time on the screen as fluorescence versus cycle number or temperature plot.

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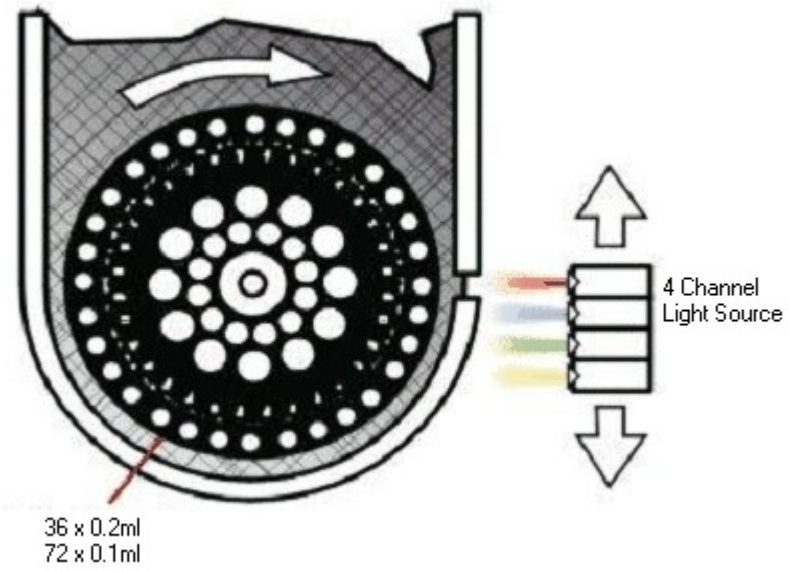
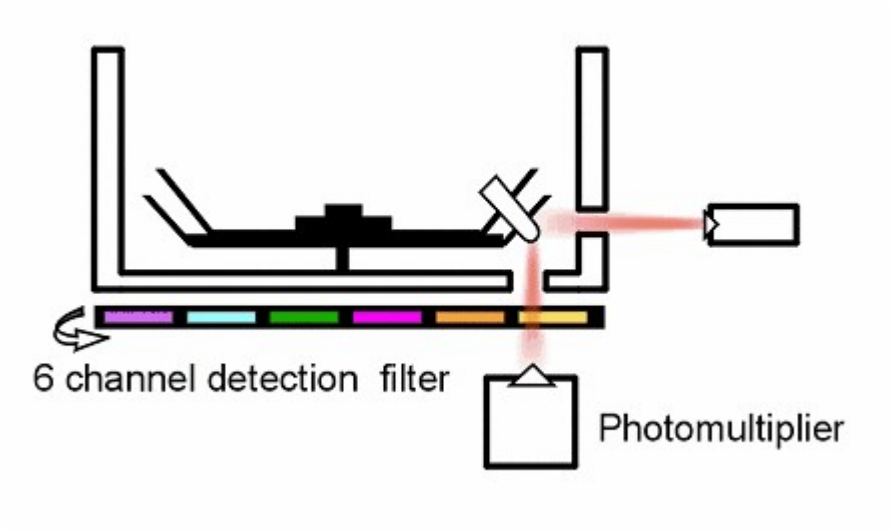
2.2 Multi-Channel Rotor-Gene

The principal of the Multi-Channel Rotor-Gene is very similar to the 32-well and the Dual-Channel Rotor-Gene. However, the Multi-Channel has one LED with four excitation filters.



From the side view of the heating cooling chamber we see the LED source irradiating the tube from the side wall and the photomultiplier detecting the energy from the base of the chamber. The detection filter wheel has six different filters, four are band-pass that are used in four Channel multiplex runs, the other two are high pass filters used with other non-standard fluorophores.



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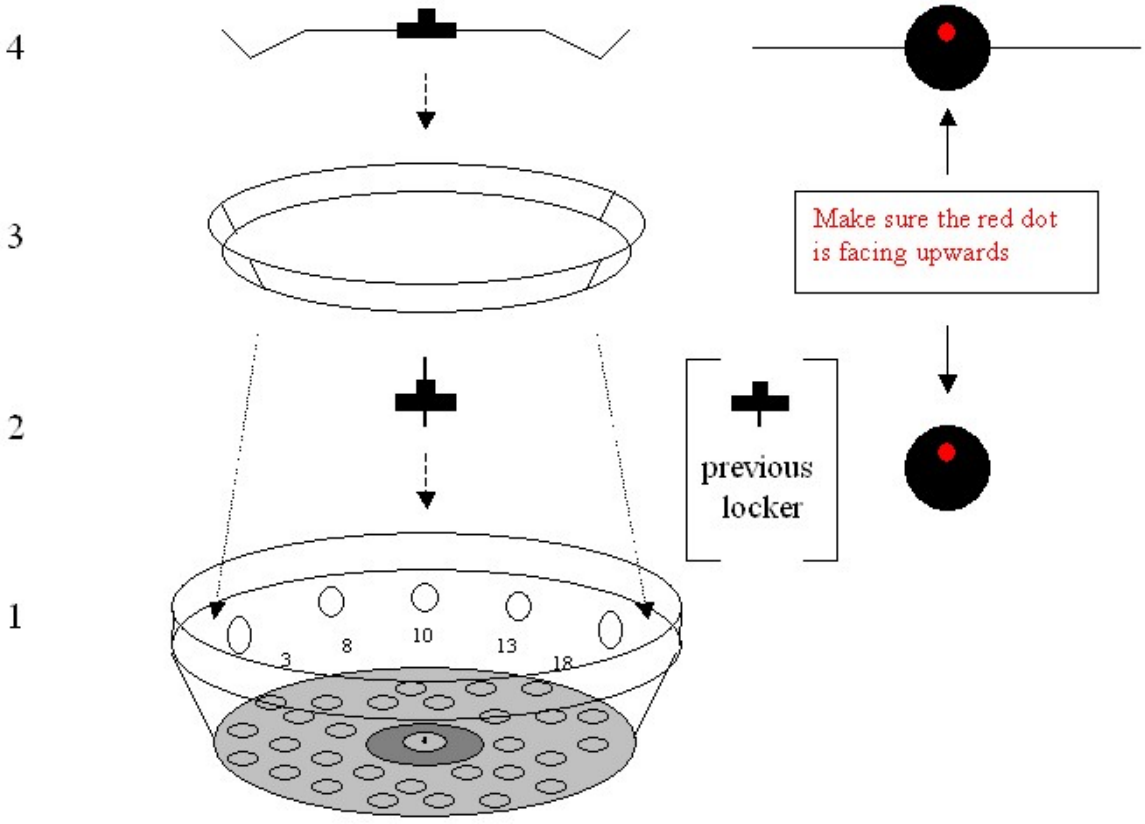
2.3 Locking Ring (For 36 Well Rotor Only)

Opening and closing 0.2 tubes several times before running might loosen the closing mechanism of tubes. The 0.2 tubes run on the 36-well rotor on the Rotor-Gene should only be closed once before running, otherwise it might result in popping of tubes. **Also make sure that the 0.2ml tubes are closed properly by firmly pressing down the lid of the tube.** If popping is experienced or wants to be avoided, a locking ring can be used on the Rotor-Gene. **The Locking Ring is designed to use for flat top 0.2 ml tubes only.**

The below graph shows schematically how the locking ring has to be used:

1) Shows the schematic representation of the 36-well rotor.
2) Screw down the screw with threads on both sides. Make sure the nipple and the red dot faces the top.
3) Simply place the locking ring over the top of the tubes.
4) Screw down the locking ring holder (4) over the screw with threads on both ends (2) until the locking ring (3) is fixed.



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3 Two Different Rotor Systems (Tubes)

3.1 Rotor 36 Well System

It is recommend that you use flat cap, 0.2ml tubes from Axygen, supplied by Corbett Research.

If using your own tubes, be aware of the following:

1. The tube cap must fit down tightly otherwise there is a risk that the cap may 'pop' open during thermal cycling. This has been observed with some domed capped 0.2ml tubes. If necessary the locking ring could be used to secure the tubes.

2. Be sure that the tubes fit into the rotor firmly. Tubes that are excessively loose may vibrate during a run and interfere with the data acquisition and give poor results.

3. In addition, ensure that the tubes are not too large. If the diameter of the tube is too large it will not fit down far enough into the rotor. Samples will not be optically aligned over the detection system and may cause a reduction in fluorescent signal acquired and sensitivity.

4. Do not use tubes from different manufacturers within the same run. This may result in inconsistencies.

5. Locking Ring can only be used with **flat top** tubes.

3.2 Rotor 72 Well System

Corbett Research manufactures the 72-well tubes and caps in strips of four especially for the Rotor-Gene.

It is not advised to autoclave the 72-well tubes and strip caps as they may distort.

The tubes can become visibly deformed and are no longer straight but bent slightly. When running tubes that have been autoclaved it has been noted that the caps may detach from the tube.
This has the effect of the loose caps being circulated around the chamber causing damage to the insulation material. It is also advisable to exchange the empty 72-well tubes after at least ten runs. Caps might start popping.

Do not use caps on the blank tubes. If caps are loaded on blank tubes and run multiple times there is a risk that caps may come off the tube causing damage to the rotor.

4 Installation and Maintenance

4.1 Installation (Rotor-Gene 2000)

The Rotor-Gene 2000 and the Desktop PC should be connected with the special "Y" mains cable supplied. This special cable minimizes the connection length between the two devices.

NOTE FOR Rotor-Gene 2000 Users: If you do not set up the mains connection as described you may see spikes in the collected data due to mains noise.

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4.2 Installation (Rotor-Gene 3000)

No special installation steps are required for the Rotor-Gene 3000. The Rotor-Gene 3000 requires only a standard serial cable to be connected to a communications port on the back of the Laptop or Desktop computer.

4.3 Maintenance

The only maintenance the user may have to perform is keeping the lenses relatively clean and free from dust that may build up over time.

It is recommended that when the Rotor-Gene is not in use, the lid is in the closed position to minimize dust falling into the heater chamber.

Looking down into the heating chamber you will notice one (36/72-Well, Multi-Channel) or two (36/72-Well, Dual-Channel) round lenses, which are the detector objectives for the different Channels. Should those lenses be covered with dust or any other debris you should clean them using a cotton bud and ethyl alcohol.

On the side-wall of the heater chamber there are also one (36/72-Well, Multi-Channel) or two (36/72-Well, Dual-Channel) round lenses for the high power diode emitter. These lenses are not likely to build up dust, however, if you place tubes into the rotor that are dirty or wet you are likely to spin those particles around the inner wall of the heater chamber and possibly produce a build up on these lenses.

If these lenses need to be cleaned, the rotor can be removed by unscrewing the black lock-down in the center and removing the rotor. These lenses can be cleaned with a cotton bud and ethyl alcohol.

5 Functional Overview

The following chapter will help to familiarize you with elements in the Rotor-Gene user interface.

5.1 Workspace

The Rotor-Gene workspace is the backdrop of the main window. This is the area in which you can open up graphs of raw data, temperature and analysis results. If you have several windows currently open, you can organize them by clicking the **Arrange button** on the toolbar. There are several additional options available, which you can access by clicking on the **Down Arrow**, which appears next to the button.

5.2 Toolbar Workspace

These buttons are shortcuts to frequently used operations. To see what their function is, hover your mouse over them and a brief explanation will appear. These commands can also be accessed via their corresponding menu items with the same name.

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5.3 View Raw Channels

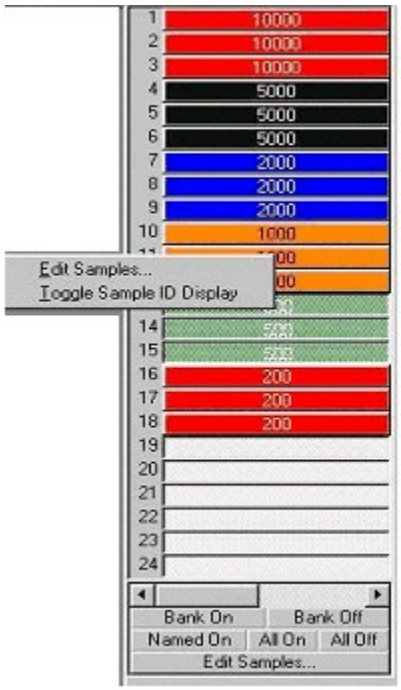
Click on these buttons to view different channels in the experiment.

5.4 Toggle Samples

Use this control to hide or show different samples. Semi-greyed samples are deselected. The **Scroll Bar** is used to display the next group of samples. You can toggle samples individually by clicking on them, or you can hide/show all currently visible (on the current 'bank') by clicking on **Bank on/ Bank off**. To select a range of samples, click on the first sample and drag your mouse to the end sample. When you release the mouse button, the selected samples will either be toggled on or off. Clicking **Named On** will only show those samples you have given a name to; a quick way to show only relevant samples. Clicking **All On /All Off** will show all/ none 36 or 72 samples. Pressing the **Edit Samples…** button opens the samples window.

Toggle samples ID display: If a 72-well rotor is used the samples are shown from A1 to A8, B1 to B8, etc. Using the toggle samples ID display button lets the user switch to a numerical order of samples (1 to 72).

The sample toggler:



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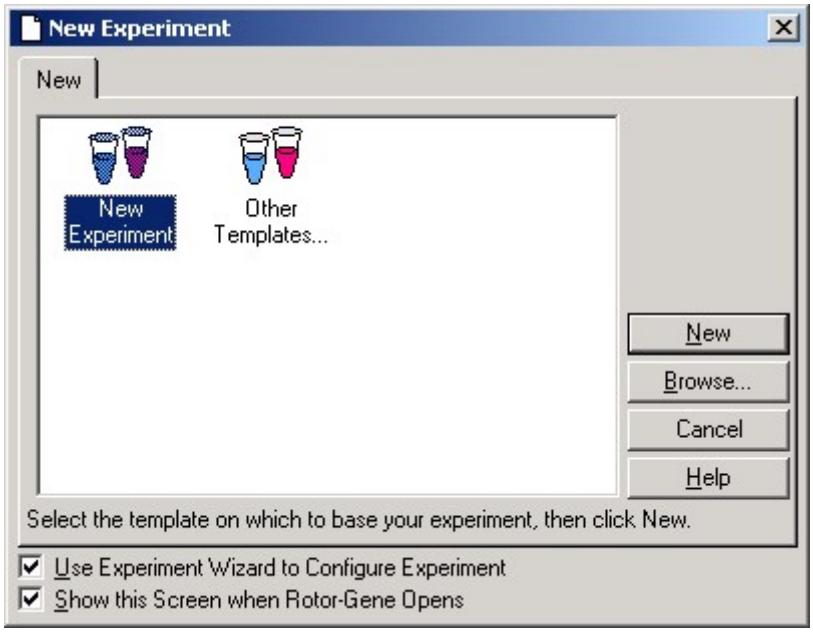
5.5 File Menu

5.5.1 New Experiment

This screen presents you with a selection of available experiment templates. In a laboratory environment, you can set up standard templates by placing them in the Rotor-Gene template directory (usually C:\Program Files\Rotor-Gene\Templates). These templates will then appear in this window.



New Experiment: Double-clicking this icon will create a new experiment based on the last experiment run. To always begin with a blank experiment, choose File, Save As and save a template in the Rotor-Gene template directory as "Blank Template.ret". Then, double-click this icon instead of New Experiment when starting a new run.

New...: Opens the template selection window to create a new experiment.

Browse...: Pressing Browse... opens a window in which you can select the template you would like to use for the next run. This option is also available under Other Templates.

Other Templates...: This icon has the same effect as clicking the Browse... button, and displays an open dialogue to load custom experiment templates.

Cancel: Closes this window.

Help: Opens on-line help.

Use Experiment Wizard to Configure Experiment: Upon selecting a template, the New Experiment Wizard will be displayed. Unticking the Use Experiment Wizard option can disable the wizard.

Show this screen when Rotor-Gene opens: Untick the box if you don't want to see this window coming up before using the Wizard.

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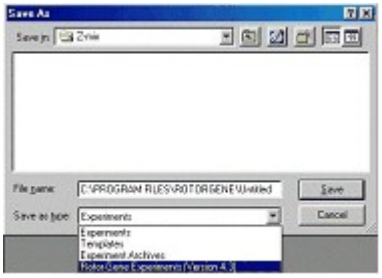
5.5.2 Opening and Saving

Open...: Opens a previously saved Rotor-Gene Experiment (REX file) or Rotor-Gene Experiment Archive (REA file).

Open Recent...: Displays the last four files you have opened or saved.

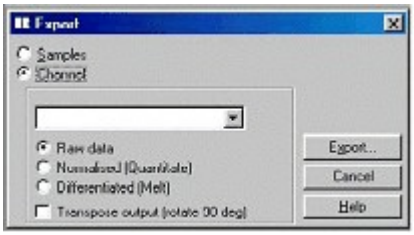
Save: Saves the experiment, prompting you for a filename if this is the first time the experiment has been run. You have the option of saving the experiment as a template, meaning that you can base future experiments on it. You can also save in the Rotor-Gene Experiment Archive (REA) format that compresses the experiment. This is ideal for emailing, as it does not need to be compressed before sending.



Save As...: Saves the experiment, always prompting you for a filename. It also lets you choose what type of file you want to save the document as.

A file can be saved as an experiment, a template, an experiment archive or an experiment in a previous RG software version (4.3).

5.5.3 Export



Opens the Export window where you can export channel and sample data to a format readable by other software. You can export Rotor-Gene data to third party software such as spreadsheet and statistical applications. This screen allows you to export channel information and the sample list.

Export Samples: The experiment's sample list will be saved in a format, which can be opened by popular spreadsheet applications. Once you have saved the samples, drag the export file into your spreadsheet.

Export Channels: You can export both raw data obtained from the machine and the data used to perform analysis after normalization and other operations have been performed. Select the channel tc export, and the type of data you wish to export, then click Export. The resultant file can be opened by dragging it into the target application.

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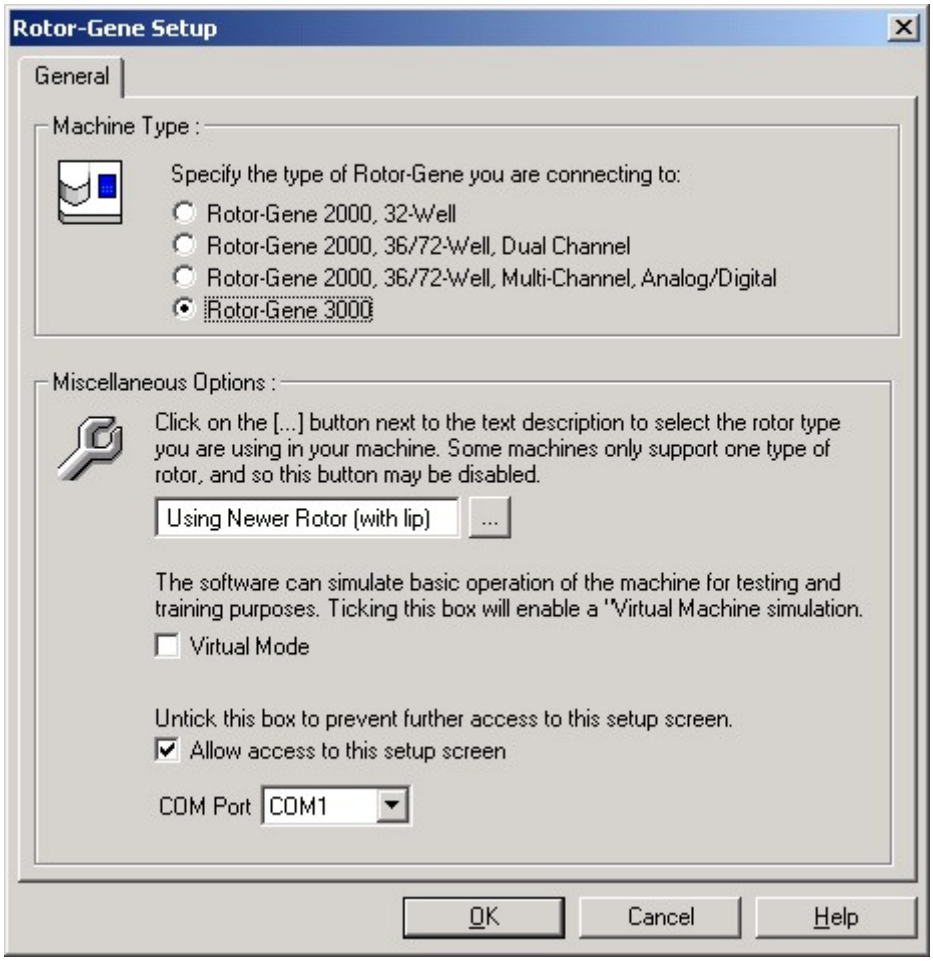
5.5.4 Setup

Choosing File, Setup... opens the Rotor-Gene setup window.


Rotor-Gene Setup
General
Machine Type :
Specify the type of Rotor-Gene you are connecting to:
Rotor-Gene 2000, 32-Well
Rotor-Gene 2000, 36/72-Well, Dual Channel
Rotor-Gene 2000, 36/72-Well, Multi-Channel, Analog/Digital
Rotor-Gene 3000
Miscellaneous Options :
Click on the [...] button next to the text description to select the rotor type you are using in your machine. Some machines only support one type of rotor, and so this button may be disabled.
Using Newer Rotor (with lip)
...
The software can simulate basic operation of the machine for testing and training purposes. Ticking this box will enable a "Virtual Machine simulation.
Virtual Mode
Untick this box to prevent further access to this setup screen.
Allow access to this setup screen
COM Port
COM1
OK
Cancel
Help

Machine Type: Select the machine you are operating from the option buttons available.

Virtual Machine: This option enables you to operate the software even when a machine is not attached for demonstration purposes.

Allow access to this screen: Untick this box, once the machine has been correctly selected to prevent users from altering this setting. If you need to have access to this screen again, contact your distributor.

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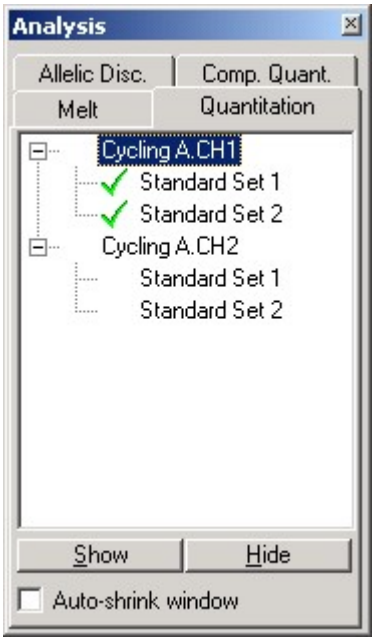
5.6 Analysis Menu

5.6.1 Analysis Toolbar

This toolbar enables you to create new analyses and displays existing ones. The tabs at the top of the bar enable you to select a method of analysis to perform. Once you have done this, a list of the channels which can be analyzed using this method will be listed below. Those that have already been analyzed will have a green tick next to them, such as the first two channels shown in the screen shot. This means that threshold and normalization settings have been remembered for this analysis. To analyze a channel, or view an existing channel, simply double-click on the channel to view. The specific analysis window will then appear.



Auto-shrink Window: Ticking the box shrinks the window when it is not used. Moving the cursor over the window enlarges the window again.

Organizing Your Workspace: Each time you double-click on a new analysis, its windows will be arranged to fit in with those already on the screen. With many windows, this can be difficult to use. Simply close the windows you do not require, then click **Arrange** on the toolbar. The windows will automatically be rearranged according to the **Smart Tiling** method. You can select another method of arrangement by clicking the **down arrow** next to the toolbar button.

Clicking the right mouse button over the analysis window also allows to **Show, Hide** or **Remove Analysis**.

5.6.2 Quantitation

Double click quantitation or press **Show** to open the channel of interest. Three windows will be opened automatically, the main screen, the standard curve and the results. Going from left to right, the following buttons are displayed in the main window:

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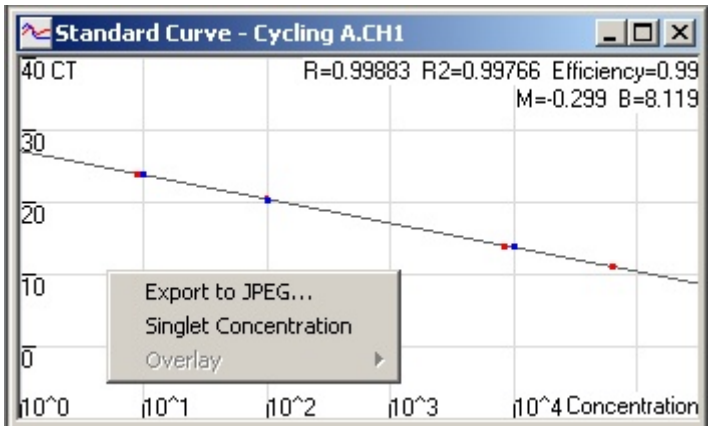
Reports

Reports: Opens the Quantitation Report selection window where you can choose a report to preview of the currently selected Quantitation analysis. There are three different options, Standard Report, Full Report and Concise Report.

Using the buttons on the top, the reports can be **printed, saved, emailed** or **exported to Word**.

Standard Curve

Std. Curve: This button opens the standard curve graph. By default, this window is opened when an analysis is opened. If you close the window, it can be re-opened by using this command.



On the standard curve the values are recalculated dynamically as the threshold level is varied, by clicking and dragging.

Blue dots on the curve show the samples that have been defined as standards and **red dots** show the unknown sample data points.

NOTE: If redefining standards to recalculate the standard curve, toggling the standard colors ON or OFF will remove it from standard curve calculation.

Efficiency: The Reaction Efficiency of the experiment. This value is discussed in more detail in Slope, Amplification, Reaction Efficiency**.**

R^2-value (correlation coefficient): The R^2 value, or R^2 value (as displayed with the superscript), is the percentage of the data which is consistent with the statistical hypothesis. In the Quantitation context, this is the percentage of the data which matches the hypothesis that the given standards form a standard curve. If the R^2 value is low, then the given standards cannot be easily fit onto a line of best fit. This means that the results obtained (ie. the calculated concentrations) may not be reliable. A good R^2-value is around 0.99.

NB: It is still possible to achieve a high R^2 value with a poor standard curve, if not enough

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standards have been run. The R^2 value will improve as the number of standards decreases.

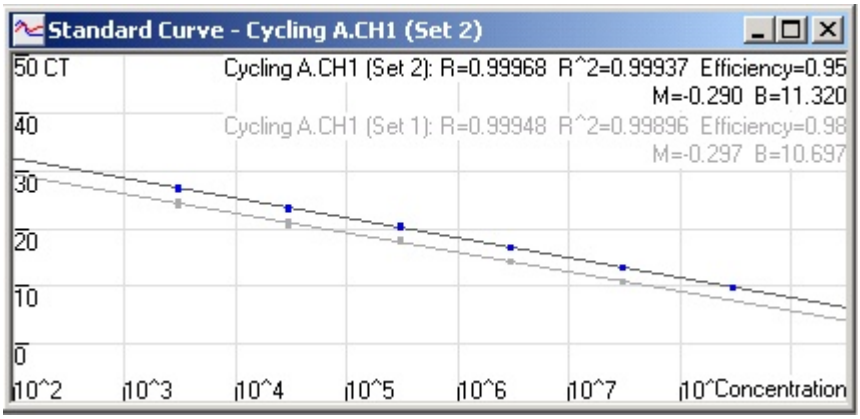
R-value (square root of correlation coefficient): The R-value of the calculation is the square root of the R^2 value. Unless you have a specific statistical application, the R2 value is more useful in determining correlation.

According to the formula **y = mx + b** the slope (**M**) and the intercept (**B**) of a standard curve are automatically calculated and shown in the top right corner of the standard curve window.

Export to JEPG...: With the pointer on the standard curve, click on the right button to show the option to export as a JEPG.

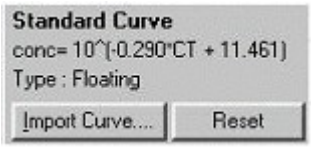
Singlet Concentration: This function is used when no replicates on a standard have been run or defined. With the function enabled the standard curve shows a line between the Given concentration and the Calculated concentration for the standard series. The threshold is then optimized, by moving the level to minimize the distance between each Given and Calculated standard concentration.

Overlay: When multiple quantitation runs have been performed in the same experiment, it is possible to overlay the standard curves in the same window. This is useful for graphically viewing the difference between different thresholds on the statistical results. Below is a screenshot of this feature:



Standard Curve Calculation

"conc=...*CT + ..." represents the equation used to relate CT values and concentrations. Type can be either floating or fixed. If floating, an optimal standard curve equation is calculated as you move the threshold. If Fixed, the equation does not change because it has been imported from another experiment.



Note: The Rotor-Gene standard curve formula is displayed in a different format to competitors' standard curves. Competitors express their curve in terms of concentrations instead of CT values, which does not affect results, but does affect the display of the M (gradient) and B (offset) values. Please read the section Why Are Rotor-Gene Standard Curves Different? for more information.

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Import Standard Curve

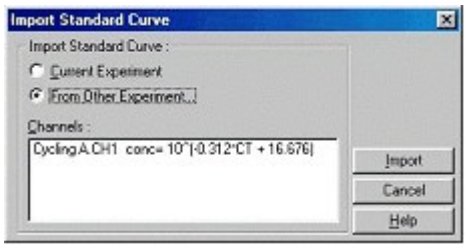
Importing a standard curve allows you to perform estimates of concentrations when a standard curve is not available in an experiment and you are certain that the reaction efficiency has not varied between the two runs. You can import curves from another channel, or from another experiment by clicking on Import Curve.

You can choose to **Adjust** the standard curve, or not to adjust. Adjusting means modifying the offset (b) in the standard curve to be consistent with the current experiment. Whether you should adjust the standard curve or not depends on the chemistry application.

A reference should be defined in the target experiment to adjust the curve to match the target experiment. You can define a reference by setting a sample's type to Standard, and entering a concentration value in the Sample Editor. Multiple copies of the same reference can be entered to improve accuracy of the method. Note that you cannot define more than 1 reference concentration. For example, it is possible to have 3 replicate references of 1000 copies, but not to have one reference of 1000 copies, and another with 100 copies in the same experiment.

Once the curve has been imported, the standard curve type will be changed to Fixed. Click **Reset** to set the curve type back to Floating.

Below is a screenshot of the Import Standard Curve screen:



Using this screen, you can import the standard curve from another channel you have analyzed in the current experiment, or you can load a standard curve from another experiment.

Current Experiment: When this option button is selected, quantitation analyses on other channels will be listed with their corresponding standard curves.

From Other Experiment: Selecting this option button will bring up an Open Dialog in which you can select an experiment to open. If any quantitation analysis has been performed for the experiment, you will see standard curves listed for each channel analyzed.

Channels List: Lists the analyzed channels and their respective standard curve formulas.

Invert Raw Data

Some chemistries produce a fluorescent readout that decreases exponentially instead of increasing. It is still possible to analyse these using Quantitation, but the "Invert Raw Data" checkbox should be ticked.

For all other quantitation analysis, this option must remain unticked.

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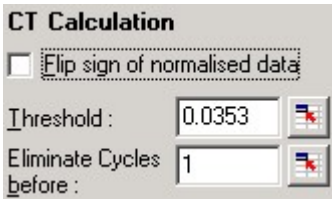
NB: Techniques such as Quenched FRET or the use of Bodipy® which use decreasing signals to quantitate may have less accurate results than those that increase. Such techniques have not been widely verified yet in the scientific community.

Calculation of CT

Ct-Calculation: The Ct value is the value where the threshold line crosses the amplification curve. By setting a threshold line and calculating the intersection with each of the sample curves, the Ct values for each sample are established.

Threshold: (Manual Set): To set the threshold click on the icon (grid with red arrow) then click and hold on the graph and drag a threshold line to the desired level, or enter a log value. Alternatively the Auto-Find Threshold function can be used to automatically determine the best level. When setting a threshold manually, it should be set in the exponential phase of the experiment, significantly above the background level to avoid noise.



Auto Find Threshold: The automatic threshold function will scan the darkened region of the graph to find a threshold setting which delivers optimal estimates of given concentrations. You can change the region to be modified by entering new upper and lower bounds in the text boxes. For most quantitation analyses, the default region is suitable. Based on the standards that have been defined the function then scans the range of threshold levels to obtain the best fit of the standard curve through the samples that have been defined as standards, (i.e. maximizes the R value to approach 1.0).

Eliminate Cycles before: To set click on the icon (grid with red arrow) then click and hold on the graph and drag the threshold line to the right. This eliminates the threshold line for low cycle numbers.

Note: This is useful when there is noise on the signal during the initial cycles.

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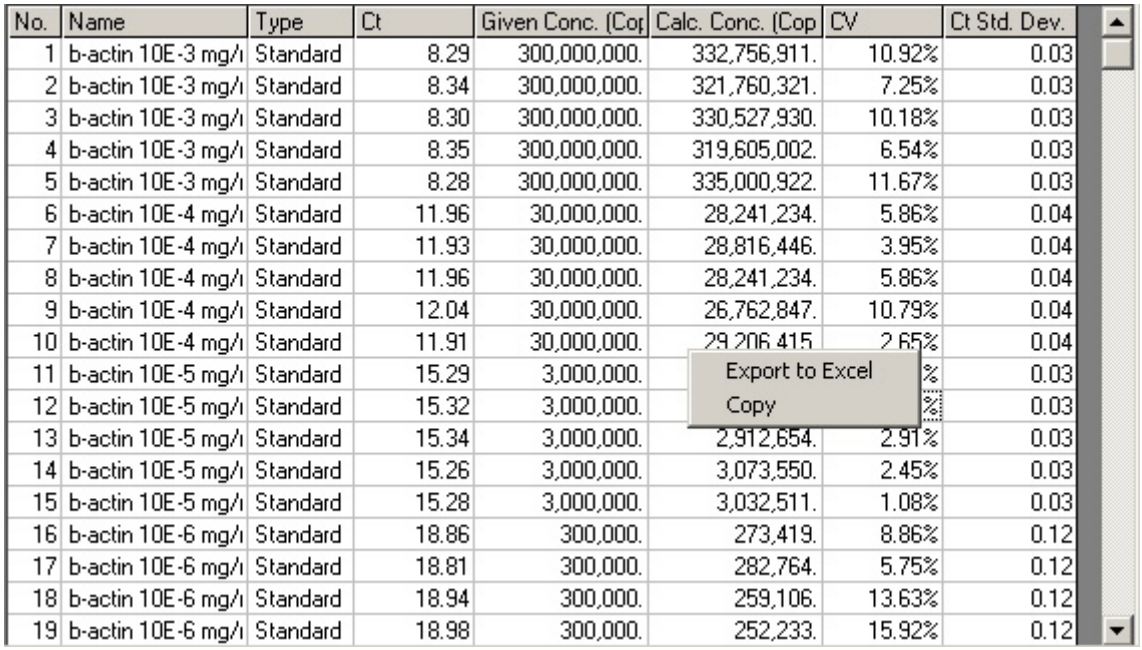
Results

Opens the quantitation results grid. By default, this window is opened when you open an analysis. If you close it, it can be re-opened by using this command.



No.	Name	Type	Ct	Given Conc. (Cop	Calc. Conc. (Cop	CV	Ct Std. Dev.
1	b-actin 10E-3 mg/l	Standard	8.29	300,000,000.	332,756,911.	10.92%	0.03
2	b-actin 10E-3 mg/l	Standard	8.34	300,000,000.	321,760,321.	7.25%	0.03
3	b-actin 10E-3 mg/l	Standard	8.30	300,000,000.	330,527,930.	10.18%	0.03
4	b-actin 10E-3 mg/l	Standard	8.35	300,000,000.	319,605,002.	6.54%	0.03
5	b-actin 10E-3 mg/l	Standard	8.28	300,000,000.	335,000,922.	11.67%	0.03
6	b-actin 10E-4 mg/l	Standard	11.96	30,000,000.	28,241,234.	5.86%	0.04
7	b-actin 10E-4 mg/l	Standard	11.93	30,000,000.	28,816,446.	3.95%	0.04
8	b-actin 10E-4 mg/l	Standard	11.96	30,000,000.	28,241,234.	5.86%	0.04
9	b-actin 10E-4 mg/l	Standard	12.04	30,000,000.	26,762,847.	10.79%	0.04
10	b-actin 10E-4 mg/l	Standard	11.91	30,000,000.	29,206,415.	2.65%	0.04
11	b-actin 10E-5 mg/l	Standard	15.29	3,000,000.		%	0.03
12	b-actin 10E-5 mg/l	Standard	15.32	3,000,000.		%	0.03
13	b-actin 10E-5 mg/l	Standard	15.34	3,000,000.	2,912,654.	2.91%	0.03
14	b-actin 10E-5 mg/l	Standard	15.26	3,000,000.	3,073,550.	2.45%	0.03
15	b-actin 10E-5 mg/l	Standard	15.28	3,000,000.	3,032,511.	1.08%	0.03
16	b-actin 10E-6 mg/l	Standard	18.86	300,000.	273,419.	8.86%	0.12
17	b-actin 10E-6 mg/l	Standard	18.81	300,000.	282,764.	5.75%	0.12
18	b-actin 10E-6 mg/l	Standard	18.94	300,000.	259,106.	13.63%	0.12
19	b-actin 10E-6 mg/l	Standard	18.98	300,000.	252,233.	15.92%	0.12

The results obtained from the experiment are summarized in a table. Clicking the right mouse button, and selecting **Export to Excel** will export the table to Excel. There is no need to open the Excel program, as this will be done automatically.If you would like to copy the data into an existing spreadsheet, choose the **Copy** option instead, then open your spreadsheet, then select paste.

To make calculations easier, a feature called **AutoStat** is introduced which automatically calculates the Average, Standard deviation, Minimum and Maximum values of samples of interest. Simply move the mouse over the area of interest and the values are given in a small table displayed below the sample list on the right-hand side of the screen.

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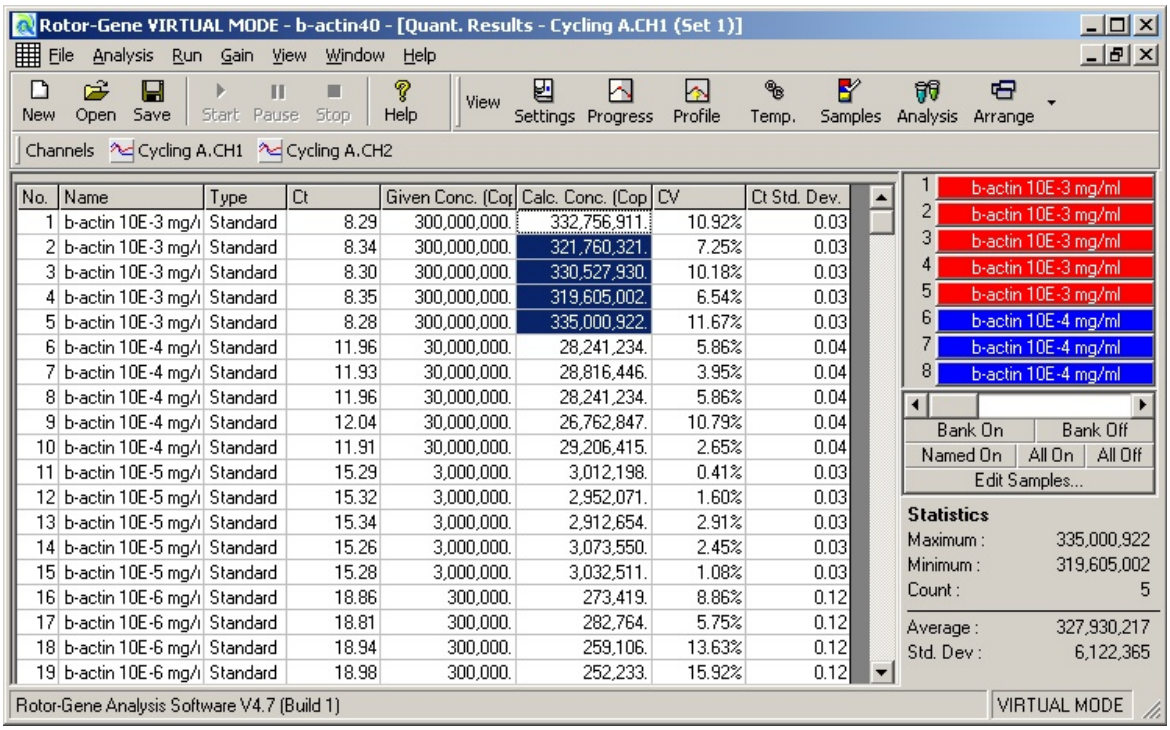
In this screenshot, samples 1 to 5 are analysed:


Rotor-Gene VIRTUAL MODE - b-actin40 - [Quant. Results - Cycling A.CH1 (Set 1)]
File Analysis Run Gain View Window Help
New Open Save Start Pause Stop Help View Settings Progress Profile Temp. Samples Analysis Arrange
Channels Cycling A.CH1 Cycling A.CH2
No. Name Type Ct Given Conc. (Cop Calc. Conc. (Cop CV Ct Std. Dev.
1 b-actin 10E-3 mg/l Standard 8.29 300,000,000. 332,756,911. 10.92% 0.03
2 b-actin 10E-3 mg/l Standard 8.34 300,000,000. 321,760,321. 7.25% 0.03
3 b-actin 10E-3 mg/l Standard 8.30 300,000,000. 330,527,930. 10.18% 0.03
4 b-actin 10E-3 mg/l Standard 8.35 300,000,000. 319,605,002. 6.54% 0.03
5 b-actin 10E-3 mg/l Standard 8.28 300,000,000. 335,000,922. 11.67% 0.03
6 b-actin 10E-4 mg/l Standard 11.96 30,000,000. 28,241,234. 5.86% 0.04
7 b-actin 10E-4 mg/l Standard 11.93 30,000,000. 28,816,446. 3.95% 0.04
8 b-actin 10E-4 mg/l Standard 11.96 30,000,000. 28,241,234. 5.86% 0.04
9 b-actin 10E-4 mg/l Standard 12.04 30,000,000. 26,762,847. 10.79% 0.04
10 b-actin 10E-4 mg/l Standard 11.91 30,000,000. 29,206,415. 2.65% 0.04
11 b-actin 10E-5 mg/l Standard 15.29 3,000,000. 3,012,198. 0.41% 0.03
12 b-actin 10E-5 mg/l Standard 15.32 3,000,000. 2,952,071. 1.60% 0.03
13 b-actin 10E-5 mg/l Standard 15.34 3,000,000. 2,912,654. 2.91% 0.03
14 b-actin 10E-5 mg/l Standard 15.26 3,000,000. 3,073,550. 2.45% 0.03
15 b-actin 10E-5 mg/l Standard 15.28 3,000,000. 3,032,511. 1.08% 0.03
16 b-actin 10E-6 mg/l Standard 18.86 300,000. 273,419. 8.86% 0.12
17 b-actin 10E-6 mg/l Standard 18.81 300,000. 282,764. 5.75% 0.12
18 b-actin 10E-6 mg/l Standard 18.94 300,000. 259,106. 13.63% 0.12
19 b-actin 10E-6 mg/l Standard 18.98 300,000. 252,233. 15.92% 0.12
1 b-actin 10E-3 mg/ml
2 b-actin 10E-3 mg/ml
3 b-actin 10E-3 mg/ml
4 b-actin 10E-3 mg/ml
5 b-actin 10E-3 mg/ml
6 b-actin 10E-4 mg/ml
7 b-actin 10E-4 mg/ml
8 b-actin 10E-4 mg/ml
Bank On Bank Off
Named On All On All Off
Edit Samples...
Statistics
Maximum : 335,000,922
Minimum : 319,605,002
Count : 5
Average : 327,930,217
Std. Dev : 6,122,365
Rotor-Gene Analysis Software V4.7 (Build 1)
VIRTUAL MODE

Dynamic Tube Normalisation

This option is ticked by default and is used to determine the average background of each individual sample just before amplification commences. Standard Normalization simply takes the first five cycles and uses this as an indicator for the 'background' level of each sample. All data points for the sample are then divided by this value to normalize the data. This process is then repeated for all samples. This can be inaccurate as for some samples the background level over the first five cycles may not be indicative of the background level just prior to amplification. Dynamic Tube Normalization uses the second derivative of each sample trace to determine a starting point for each sample. The background level is then averaged from cycle 1 up to this starting cycle number for each sample. **This method gives the most precise quantitation results.** Alternatively with some data sets it may be necessary to disable the dynamic tube normalization. If this is the case the average background for each of the samples is only calculated over the first 5 cycles. If the background is not constant over the cycles before amplification it will result in less precise data.

Noise Slope Correction

The background fluorescence (Fl) of a sample should ideally remain constant before amplification. However, sometimes the Fl-level can show an increase or decrease due to the effect of the chemistry being run and produce a skewed noise level. The Noise Slope Correction option uses a line-of-best-fit to determine the noise level instead of an average, and normalizes to that instead. Turning on this option can tighten replicates if your sample baselines are noticeably sloped.
This function improves the data when raw data backgrounds are seen to slope upward or downward before the amplification take off point (Ct). It is very helpful for experiments when for example the FAM background is seen to creep upwards over the initial cycles.

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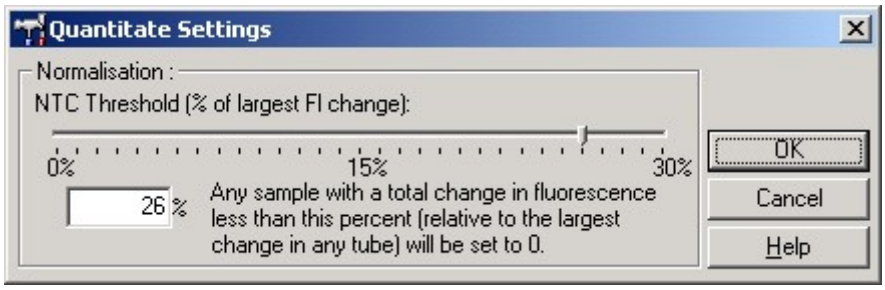
Ignore First

The first couple of cycles in a quantitation run are not usually representative of the rest of the experiment. For this reason, you may get better results if you select to ignore the first few cycles. If the first cycles look similar to cycles after them, you will gain better results by disabling this function, as the normalization algorithm will have more data to work with. You can ignore up to ten cycles.

Quant. Settings

The quantitation setting in the quantitation screen allows excluding samples or NTCs, which have a slight drift upwards, due to probe degradation or other effects. All samples with a change below the NTC threshold will not be reported in the quantitation screen. The percentage is relative to the largest maximum change found in any tube. For example, if you have one sample which began at a background of 2Fl and went to 47Fl, then 45Fl represents 100%. An NTC threshold of 10% would consider as noise any sample with a reaction less than 4.5Fl.


Quantitate Settings
Normalisation :
NTC Threshold (% of largest Fl change):
0%
15%
30%
26 %
Any sample with a total change in fluorescence less than this percent (relative to the largest change in any tube) will be set to 0.
OK
Cancel
Help

Slope, Amplification, Reaction Efficiency

The slope (m) of a reaction (shown in the standard curve window), once slightly transformed, can be used to determine the exponential amplification and efficiency of a reaction.

Calculate a gradient g = 1 / m. This gradient g is used in the following equations, and not the m value. This is because the slope used in the Rotor-Gene software and in other applications is reported in a different but equivalent manner. The reason for this is explained in the topic "Why are Rotor-Gene Standard Curves different?".

The following calculations give some important results: $\text{exponential amplification} = 10^{(-1/g)}$ or $\text{reaction efficiency} = [10^{(-1/g)}] - 1$. Optimal values for g, amplification and reaction efficiencies are –3.322, 2 or 1, respectively. The efficiency of a reaction is reported in the quantitation report.

The slope is calculated of being the change in Ct divided by the change in log input (for example copy number). A 100% efficient amplification means a doubling of amplification product in each cycle resulting in a g value of -3.322, an amplification factor of 2 and a reaction efficiency of 1.

Given a g value of –3.322, the calculations are as follows:

Amplification value: $10^{(-1/-3.322)} = 2,$
Reaction efficiency: $[10^{(-1/-3.322)}] - 1 = 1.$

Here are two examples for two different slope values.

A g value of 3.8 means that the reaction has an amplification value of ~1.83 and a reaction efficiency of 0.83 (or 83%).
There could be several reasons for this value. If the value needs to be improved, optimization steps like

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primer or probe concentrations, $MgCl_2$- or Sybr-Green I concentrations could be improved.

A g value of 3 means that the reaction is more than 100% efficient. A reason for this could be an unproportional digestion of probe compared to the amplicon produced. In addition, if the R-value is low, then statistical error can cause an unexpected reaction efficiency.

Offset

Intercept:
In a formula describing the relation between two variables, the intercept is expressed with the letter "b" (y = mx + b). The intercept is also sometimes referred to as the **Offset**.

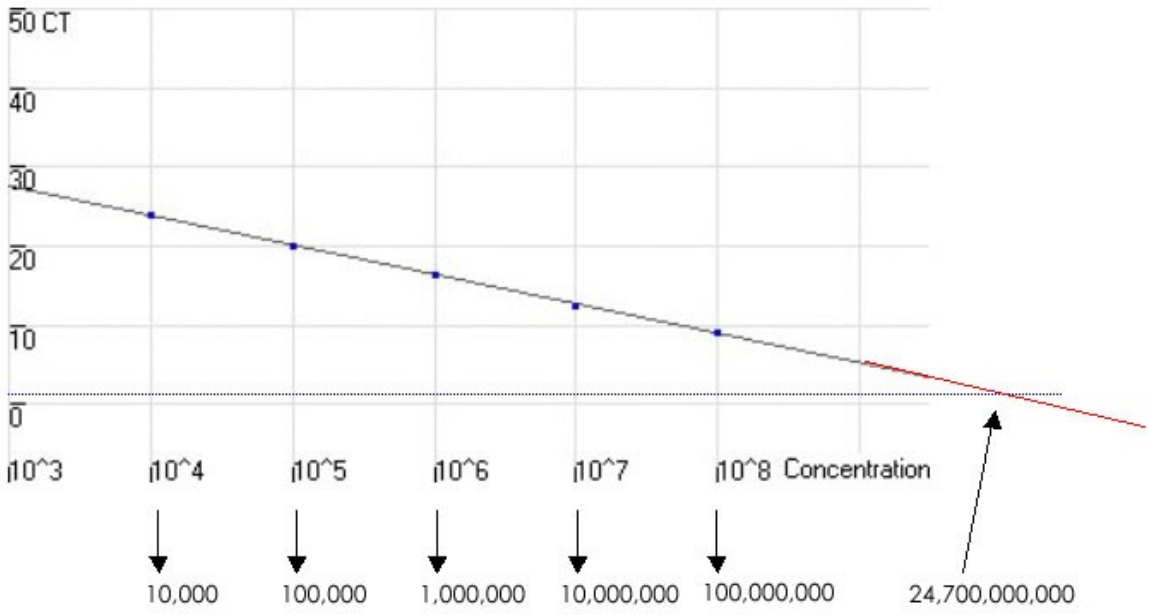
The intercept used in the standard curve of the Rotor-Gene software is the maximum theoretical number of copies that could have been detected at cycle number 1.

Example:
The below shown standard curve has the following values:
R = 0.99977
M = 0.268
B = 10.393

A dilution series has been performed starting with a copy number of 10^0 copies. Four dilutions have been performed down to 10^4 copies.
The intercept in this example is B = 10.393. The doted blue line below represents a CT-value of 1 and the red line is an extrapolation of the standard curve. The crossing point of those two lines gives the maximal theoretical amount of copies which could have been detected. The calculation is as follows:

Calculation:

$10^{10.393} = 24{,}700{,}000{,}000$



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Why Are Rotor-Gene Standard Curves Different?

The short answer is that the Rotor-Gene standard curves and the competitor standard curves display exactly the same information, but in a different manner. If you are not satisfied with this explanation, then this section is of interest to you.

Many competitors provide a standard curve as a linear function from concentrations to CT values. This means, the standard curve formula they produce allows to you to find out the CT value for a sample if you know its concentration. The Rotor-Gene contains a formula written the other way around. That is, given a CT value, tell me the concentration. Of course, this is exactly what the Quantitation process is all about -- finding out concentrations of samples given their CT values. This is the reason we chose this method of representing the information. The different approaches of the Rotor-Gene and competitors has, however, been the source of some confusion.

This is the relationship between the values:

"competitor m value" = 1 / "Rotor-Gene m value"
"competitor b value" = -"Rotor-Gene b value" * "competitor m value"

Here is the proof of the correlation between the Rotor-Gene and Competitor standard curves. It is a basic rearrangement of two functions.

Let rm, rb be the Rotor-Gene gradient and offset.
Let cm, cb be the Competitor gradient and offset.
Let Concentration be the calculated concentration for a sample.
Let CT be the CT value for a sample.

Now,

Log(Concentration) = rm * CT + rb *(Rotor-Gene has a function from CTs to Concentrations)*

and

CT = cm * Log(Concentration) + cb *(Competitors have a function from concentrations to CTs)*

Then,

Log(Concentration) = rm * CT + rb *(rearranging)*
(Log(concentration) - rb) / rm = CT
(Log(concentration) - rb) * 1 / rm = CT

Let f = 1 / rm

(Log(concentration) - rb) * q = ct
(Log(concentration) - rb) * q = ct
Log(concentration) * q - rb * q = ct *(refactoring)*

let g = -rb * f

Then

CT = Log(Concentration) * f + g

Which is exactly the same as the competitor equation, by substituting
f == cm and g == cb.

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Thus,

cm = 1 / rm and cb = -rb * cm

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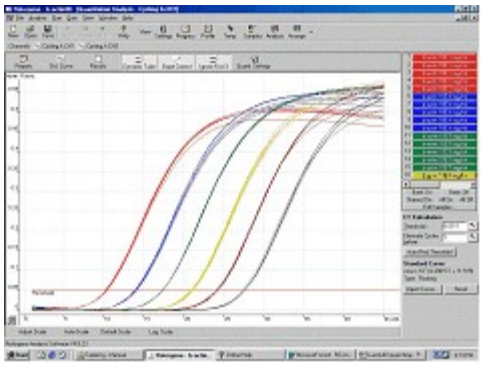
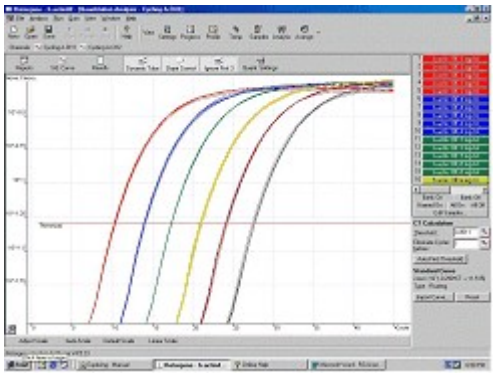
Main Window

This screen shows that taking the average background energy for each of the samples normalizes the data which is then converted to a Log scale.

From this Log graph a threshold can be determined to calculate the Ct value for each of the samples. The Ct value denotes the starting cycle for the amplification. This Ct value can be related directly to the starting copy number of the sample.





Pressing **Linear Scale** on the bottom of the screen takes you directly from the **Log Scale** to the **Linear Scale** or the other way round.
The difference between the Linear and the Log Scale is only how the data is presented. The lower values in the Log-Scale are "blown up" to make data readable easier. The Log Scale emphasizes the normalized data approaching zero. The linear scale is the normalized RAW data, we see all samples with the same starting background.
The log-scale is the LOG of the normalized data, we see the small increase at the take off point expanded so we can accurately determine the Ct value. This could easily be checked by turning on the pinpointer in both graphs and compare the numbers obtained for the fluorescence. It can be seen that there is no difference between the actual data.

Note: This process can also be performed while the Rotor-Gene is running. This real-time monitoring of quantitation data provides the user with the possibility to gain results as soon as the curves show an exponential growth. Preliminary conclusions could be drawn already and decisions made for the next run.

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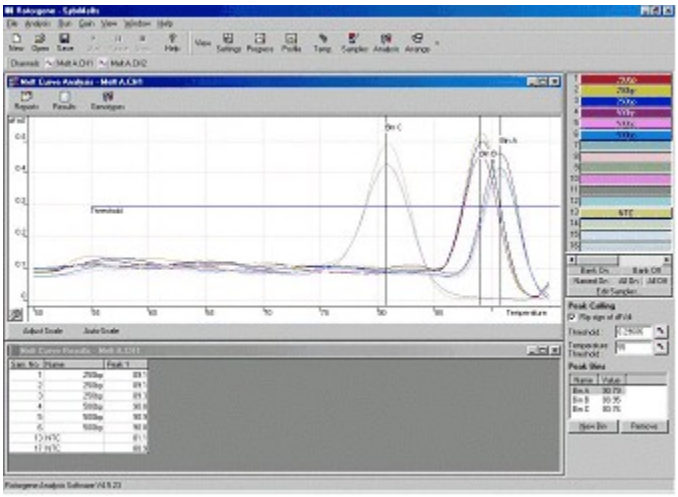
5.6.3 Melt Curve Analysis

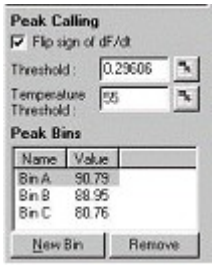
Melt Curve analysis analyses the derivative of the raw data, after smoothing. You can use it to perform genotyping, to find take-off points in reactions and numerous other applications. Peaks in the curve are grouped into bin groups, and all peaks below the threshold are discarded. You then map bins to genotypes through the Genotype, Edit Genotypes menu.

Typically after a cycling run has been finished a melt step can be added to visualize the melt characteristics of the products. The sample temperature is increased at a linear rate and the fluorescence of each sample is recorded.

Below is a typical melt curve analysis shown for a Sybr Green I amplification.



Sidebar



Flip sign of dF/dT: Before defining peaks ensure the dF/dT sign is correct for the data set to give positive peaks.

Defining Peaks: In the Melt Curve Analysis screen peaks can be defined and reported using different methods. One is to automatically call all the peaks for each sample. The other is to assign peaks to bins, which is useful for genotyping samples.

Any peak that is within a range of +/-2°C of the bin center will be assigned to the bin. If there are two peak bins close together then the peak will be assigned to the closest bin.

Bins are used to define the general area where you expect peaks to occur. The melt analysis software clusters peaks into bin groups, based upon actual peak values in the curve.

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Note: The peak bins should not be used for estimating peak positions, use the Automatic Peak Calling table instead for this purpose.

Peak Bins: To define a peak bin click on the **New Bin** button, then click and hold on the graph to define the center of the peak bin. To add another bin repeat the process or use the **Remove** button to delete peak bins.

Threshold Level: To set the threshold, click on the icon (grid with red arrow) then click and hold on the graph and drag a threshold line to the desired level.

Temperature Threshold: To set click on the icon (grid with red arrow) then click and hold on the graph and drag the threshold line to the right. This eliminates the threshold line for the lower temperatures.

Note: This is useful when there is noise on the signal at low temperatures.

Reports

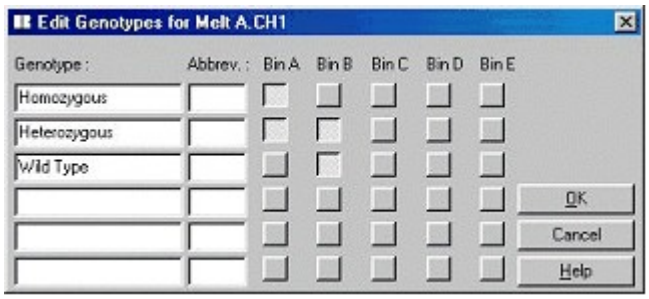
Opens the Melt Report selection window where you can choose a report to preview. You can generate a report based upon the currently selected channel, or you can generate a Multi-Channel genotyping report.

Results

Displays the result grid, showing peaks in samples.

Genotyping

Click on the genotyping toolbar and select the genotype definitions.



This screen lets you assign genotypes to the incidence of peaks in bins. The default genotype configuration is shown in the screenshot, with heterozygous samples having two peaks, homozygous a peak in the first and wild type samples in the second bin. Next to the name of each genotype is a field for typing in an abbreviation. This is used when printing multi-channel genotyping reports so that all results from multiple channels can be read easily across the screen.

For multiplex analysis, genotypes have to be setup in each channel. If for example a dual channel FRET analysis is run, where a wild type, heterozygous and homozygous is expected in each channel, the above setup has to be performed for each channel. The results will than be given in a multiplex report.

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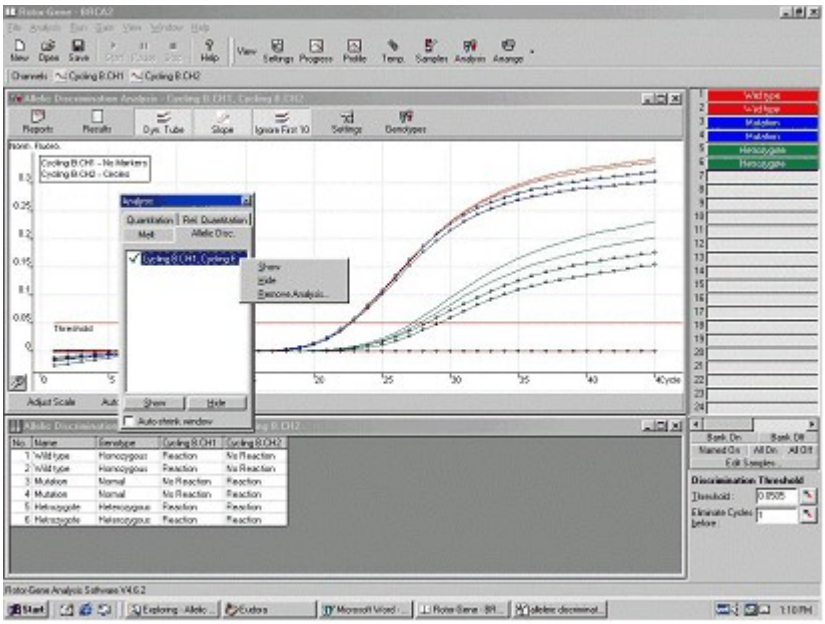
5.6.4 Allelic Discrimination

For Allelic Discrimination, it is not sufficient to double-click on the channel you would like to analyze, as this analysis is performed using multiple channels simultaneously. To perform this analysis, either hold down SHIFT and click to highlight each channel you wish to analyze, or drag your mouse over these channels. Once the desired channels have been highlighted, click **Show**. The list will update to show all the channels on one line, with a tick next to them. This indicates that they are all being used in one analysis. You can "break apart" these channels by right clicking on the analysis and selecting **Remove Analysis...** You will then be able to include those channels in another Allelic Discrimination analysis. Please note that a channel can only be used once in each type of analysis.

Allelic Discrimination Analysis allows you to perform genotyping using dual labeled probes. The genotype of samples is displayed in the result window.



Reports

Opens the Allelic Discrimination Report for preview.

Results

Displays the genotyping results grid. This grid is opened when the analysis is first displayed by default.

Normalisation Options

A variety of options are available to optimise the way in which the data is normalised:

- Dynamic Tube (Dynamic Tube Normalization)
- Slope Correct (Noise Slope Correction)
- Ignore First x (First x Cycles Noise Correction)

These terms are explained in the chapter Quantitation.

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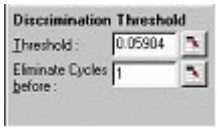
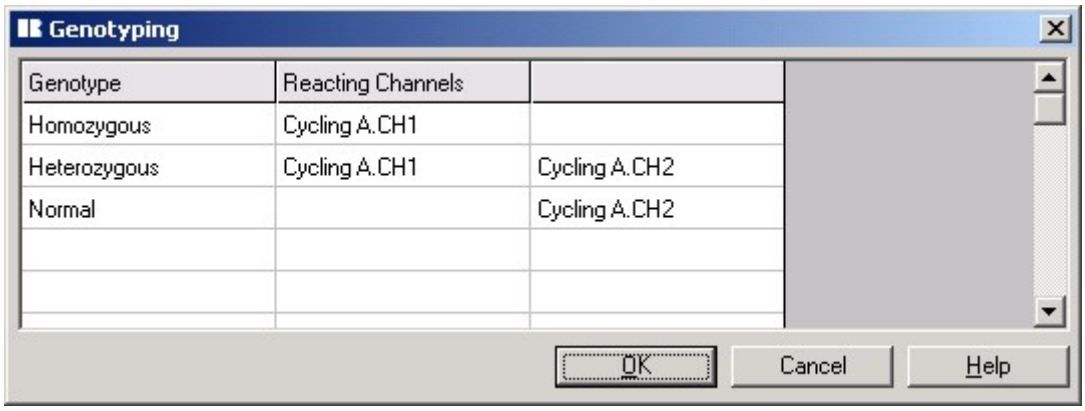
Discrimination Threshold



Discrimination Threshold: Enter values in these text boxes to position the discrimination threshold. All curves surpassing this line will be considered as having amplified for the purposes of genotyping. Click on the button to the right of each text box then drag the threshold on the graph to set these values visually.

Genotypes

Genotypes: Opens the genotype window to define which genotype is detected in which channel.



This screen lets you assign genotypes to reacting channels for an Allelic Discrimination analysis. In the above example, a sample is heterozygous if readings in channels Cycling A.CH1 and Cycling A.CH2 cross the threshold.

5.6.5 Scatter Analysis

For Scatter Analysis, it is not sufficient to double-click on the channel you would like to analyze, as this analysis is performed using multiple channels simultaneously. To perform this analysis, either hold down SHIFT and click to highlight each channel you wish to analyze, or drag your mouse over these channels. Once the desired channels have been highlighted, click **Show**. The list will update to show all the channels on one line, with a tick next to them. This indicates that they are all being used in one analysis. You can "break apart" these channels by right clicking on the analysis and selecting **Remove Analysis...** You will then be able to include those channels in another Scatter Analysis analysis. Please note that a channel can only be used once in each type of analysis.

Scatter Analysis allows you to observe how different samples will react using different channels, reactions are graphed on a scatter plot allowing a visual comparison across both channels.

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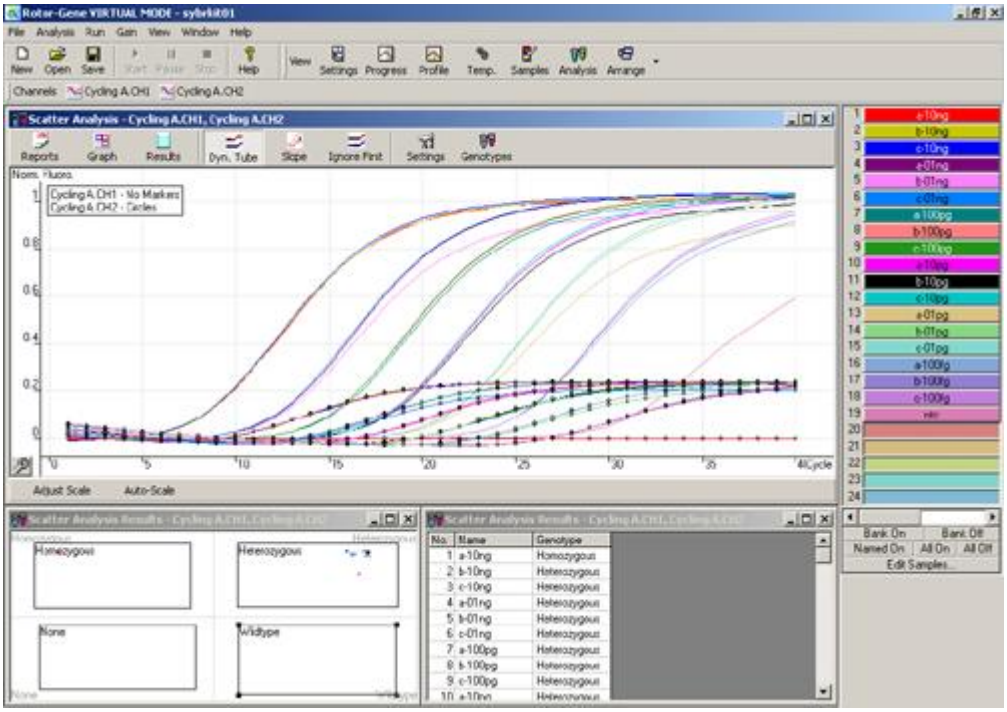

Reports

Opens the Scatter Analysis Report for preview.

Results

Displays the genotyping results grid. The genotype for each sample is determined by the custom regions which the user may defined using the scatter graph.

Normalisation Options

A variety of options are available to optimise the way in which the data is normalised:

- Dynamic Tube (Dynamic Tube Normalization)
- Slope Correct (Noise Slope Correction)
- Ignore First x (First x Cycles Noise Correction)

These terms are explained in the chapter Quantitation.

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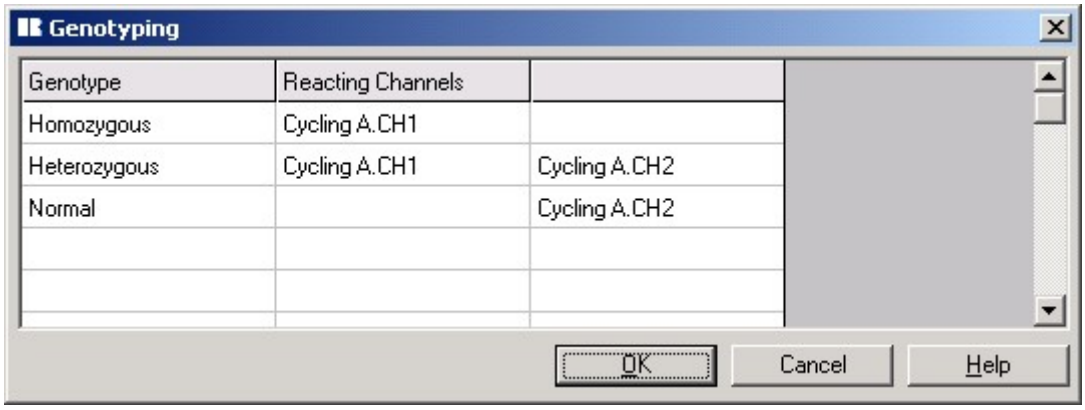
Genotypes

Genotypes: Opens the genotype window to define which genotype is detected in which channel.



This screen lets you assign genotypes to reacting channels for a scatter analysis. Defining a genotype here, gives you the option to define a user-defined genotype on the scatter graph. Note that it is the user-defined genotypes from the scatter graph that will determine the results, default genotypes defined here are for display purposes only and will appear in the appropriate corners of the scatter graph.

Scatter Graph

The scatter graph is a plot of both channels on their respective axes, giving a visual comparison so we can easily see how the sample react on different channels.

The user is given the option to define custom genotypes by clicking on the graph and dragging to define a region. The regions that are available can be modified through the Genotype screen.

5.6.6 Comparitive Quantitation

The feature "**Comparative Quantitation**" is used to compare the relative expressions of samples to a control sample in a run when a standard curve is not available. Users testing results from Microarray analysis frequently use this feature.

To perform the analysis, go to **Analysis** and select **"Comp. quantitation"**. Double-click on the channel to analysis. Chose a control sample by using the pull down menu on the right-hand side of the

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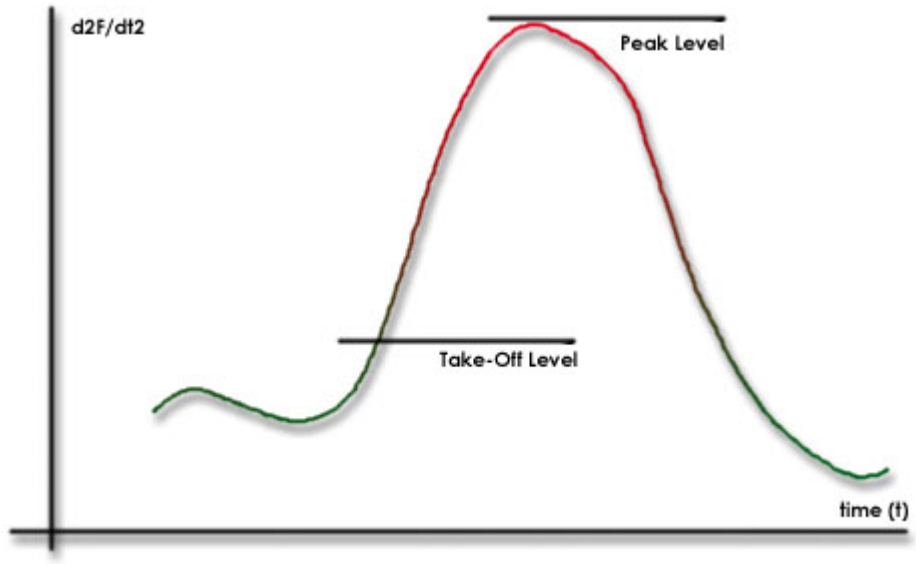
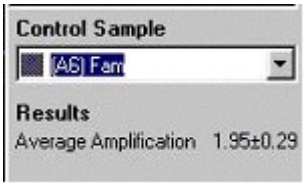
screen below the sample toggler. The table below the graph will automatically calculate the results.

The first column of the table shows the names of the samples. The second column is called "Take off" and gives the take off point of the samples. To calculate the take off point, the second derivative of the raw data is taken. Then, a peak is found where the reaction is increasingly most rapidly. This is the peak of the exponential reaction and occurs shortly after the take-off of the reaction. The take-off point cannot be determined exactly, but is estimated by finding the first point to be 80% below the peak level.

This graph shows a second derivative of a quantitation reaction, showing the peak, and the 20% level used to indicate the level:



The third column gives the efficiency of the particular sample. A 100% efficient reaction would result in an amplification value of 2 for every sample, which means that a doubling of an amplicon takes place in every cycle. In terms of the raw data, the signal should increase by a doubling amount in the exponential phase. So, if the signal was 50 at cycle 12, then went to 51 at cycle 13, it should go to 53 fluorescence units at cycle 14. All of the amplification values for each sample are averaged to produce the amplification value that is shown on the right-hand side of the screen. The more variation there is between the estimated amplification values of each sample, then the larger the confidence interval will be (indicated by the value after the ± sign). The confidence interval gives a 68.3% probability that the true amplification of the samples lies within this range (1 standard deviation). By doubling the ± interval, one achieves a 95.4% confidence interval.



The average amplification is needed to calculate how much more or less a sample is expressed. If for example the amplification value was lower, a certain absolute copy number of an amplicon is obtained later than if the amplification value was higher. The last column finally gives the comparative quantitation value. Based on the take off point and the reaction efficiency it calculates the relative concentration of each sample compared to the control sample that was chosen by the user. The

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number given is expressed in scientific notation.

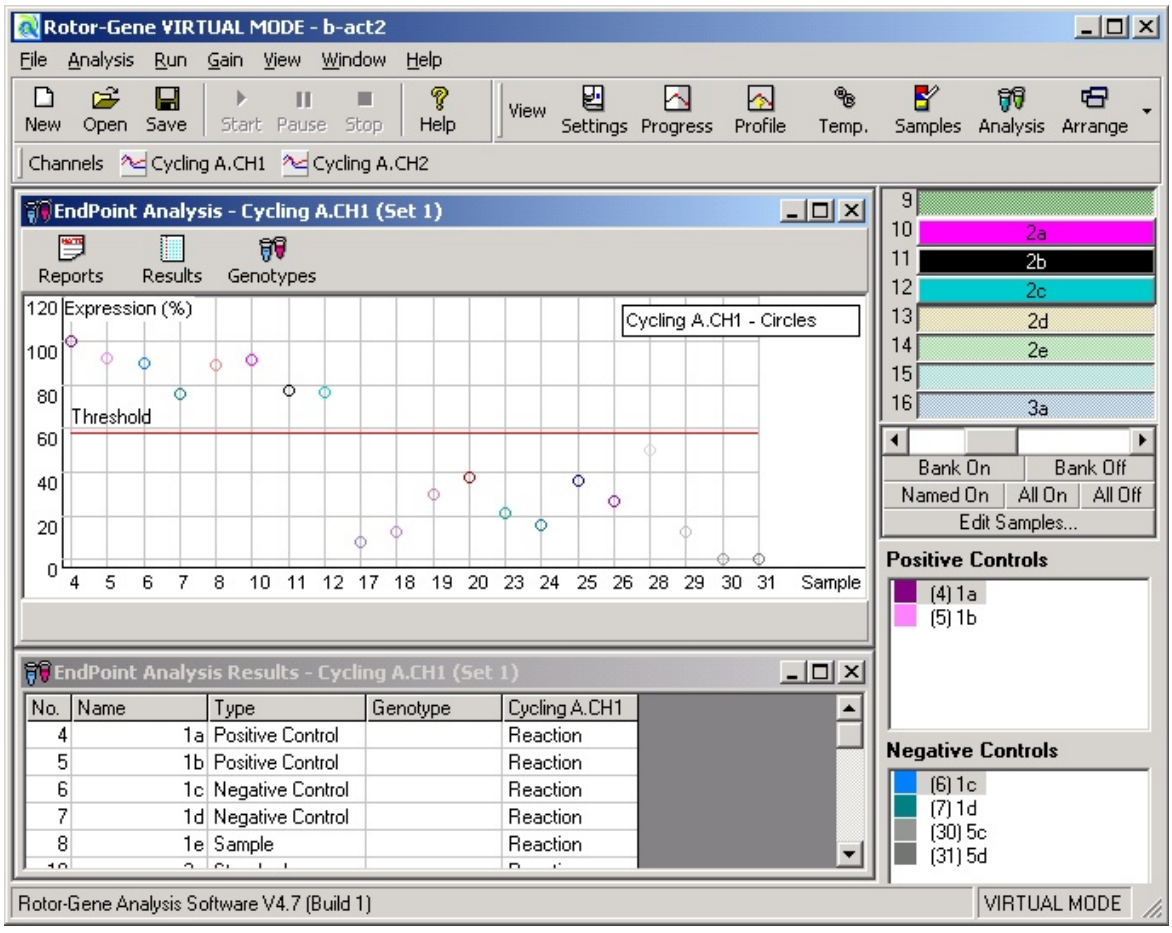
Note: If a large value is displayed after the +- in the Average Amplification, then the results are likely to be unreliable. The interval that the average amplification represents the range for which the results are valid.

Steps taken to calculate relative concentrations:
1. The take-off points of each sample are calculated by looking at the second derivative peaks.
2. The average increase in raw data 4 points following the Take-Off is calculated and becomes the sample's amplification.
3. The amplications are averaged to become an experiment "Average Amplification".
4. The relative concentration for a sample is calculated as Amplification ^ (ControlTakeOff - ThisSampleTakeOff).
5. The result is displayed in scientific notation.

5.6.7 EndPoint Analysis

EndPoint Analysis is a technique which allows the determination of relative levels of concentration for runs performed in a non-RT thermal cycler.

Below is a screenshot of the EndPoint Analysis software module:


Rotor-Gene VIRTUAL MODE - b-act2
File Analysis Run Gain View Window Help
New Open Save Start Pause Stop Help View Settings Progress Profile Temp. Samples Analysis Arrange
Channels Cycling A.CH1 Cycling A.CH2
EndPoint Analysis - Cycling A.CH1 (Set 1)
Reports Results Genotypes
Expression (%)
Cycling A.CH1 - Circles
Threshold
Sample
EndPoint Analysis Results - Cycling A.CH1 (Set 1)
No. Name Type Genotype Cycling A.CH1
4 1a Positive Control Reaction
5 1b Positive Control Reaction
6 1c Negative Control Reaction
7 1d Negative Control Reaction
8 1e Sample Reaction
Bank On Bank Off
Named On All On All Off
Edit Samples...
Positive Controls
(4) 1a
(5) 1b
Negative Controls
(6) 1c
(7) 1d
(30) 5c
(31) 5d
Rotor-Gene Analysis Software V4.7 (Build 1)
VIRTUAL MODE

EndPoint Analysis is similar to Allelic Discrimination, in that the results are of the form "reaction/no

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reaction", and that names can be assigned to certain permutations of reactions over different channels. Where EndPoint Analysis is different is that only a single reading is available instead of a cycle-by-cycle reading for each sample. This means that the user must supply additional information to help facilitate the analysis, namely, the identification of **positive** and **negative controls**.

To facilitate the reading of this information, a Normalisation process using known positives and negatives for each channel presents the samples in terms of their expression relative to this. The user then selects a percentage **level of expression** as the reaction threshold.

Terms Used In EndPoint Analysis

Below are explanations of the terms and concepts used in EndPoint Analysis:

Term	Explanation
Positive Control	A sample which is known to have reacted, and which represents the 100% expression level for samples on the same channel.
Negative Control	A sample which is known not to have reacted. This represents the 0% expression level for samples.
Threshold	A percentage level of expression above which a sample is said to have reacted. This setting must be adjusted by the user for each run.
Relative Expression	A percentage value which represents the fluorescence as the difference between the highest positive control and the lowest negative control.
Genotype	An interpretation of different permutations of reactions on different channels. For example, one could assign a genotype of "Heterozygous" to samples which reacted in both channels "FAM/Sybr" and "JOE". The genotype can also be used for reporting results of reactions with internal controls. For example, you may wish to report results such as "Inhibited", "Positive", "Negative" on the basis of whether a reaction was seen in certain channels.

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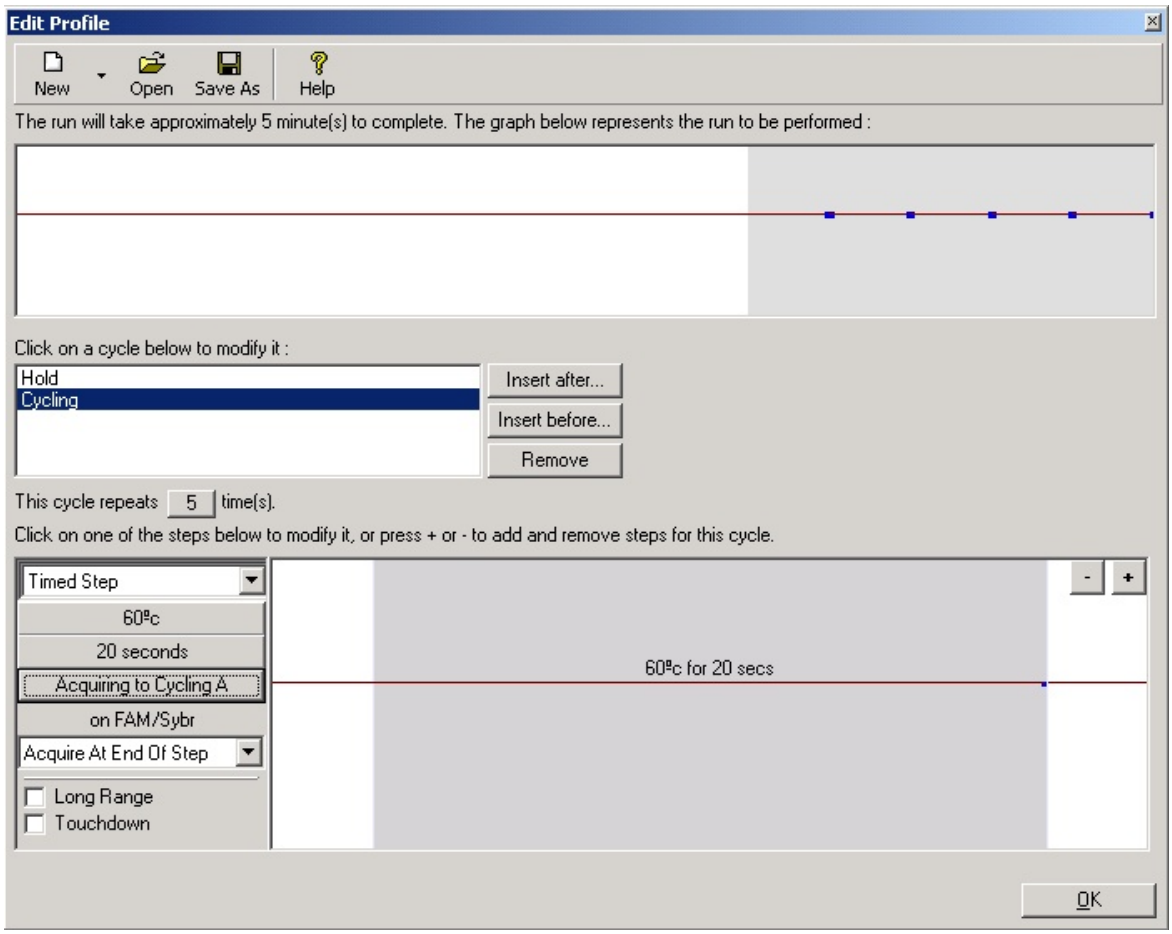
Profile Configuration

To perform an EndPoint analysis, you should cycle the samples for the appropriate time on your other thermal cycling instrument.

Once this has finished, transfer the tubes to the Rotor-Gene, and perform a profile with a hold at 60 degrees for several minutes, then a cycling step with 1 step, on 60 degrees for 20 seconds, acquiring on the required channel. Set the number of repeats to 5, as shown below:



Please note that these times are a guide only, and may vary for your particular application.

The more repeats in the profile, the more information will be available to perform the analysis. The analysis module will automatically average all the readings taken into a single value for each sample. There is no set number of repeats required, and unless you are performing an experiment where a very fine level of accuracy is required, 5 repeats should be largely sufficient.

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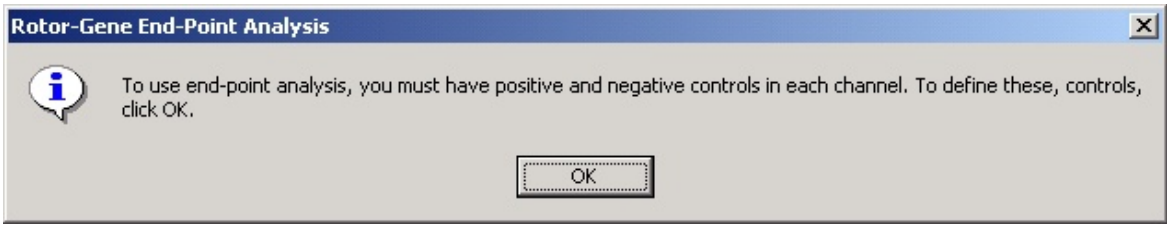
Analysis

You can perform EndPoint analysis on an number of channels simultaneously. To create a new analysis, click on the EndPoint tab, select the channels by dragging over them with your mouse, and then click **Show**. The EndPoint analysis window will be displayed.

Define Controls

When you open an EndPoint analysis for the first time, you will likely not have set up your Positive and Negative controls. The following message will be displayed :


Rotor-Gene End-Point Analysis
To use end-point analysis, you must have positive and negative controls in each channel. To define these, controls, click OK.
OK

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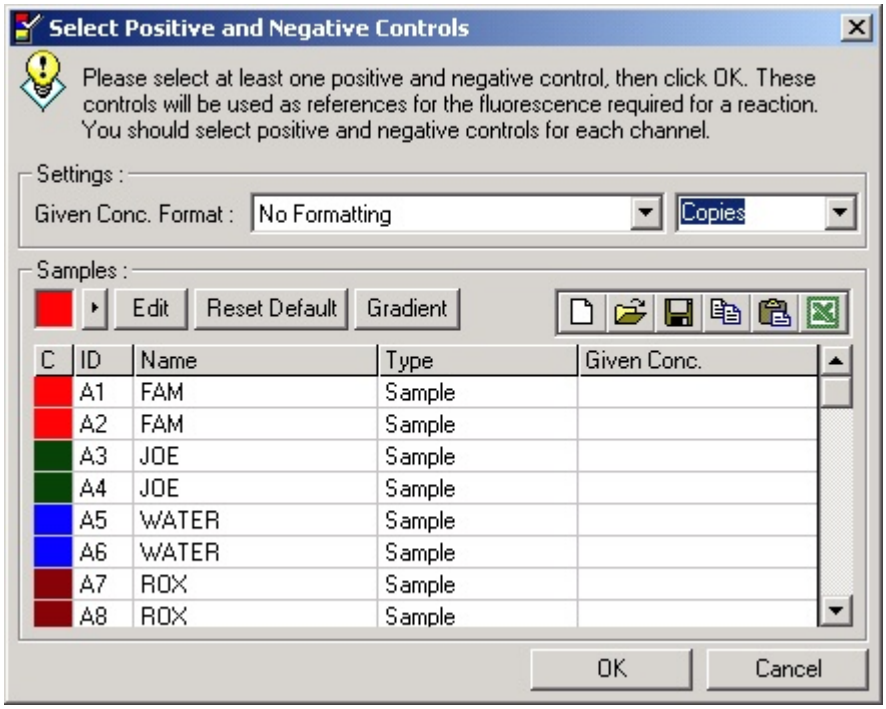
After clicking OK, the sample editor appears, allowing you to define Positive and Negative controls. To set a sample to be a Positive or Negative control, click on the cell containing the sample's type, then select the relevant control type from the drop-down box which appears next. The samples to be positive or negative controls must be turned on before you enter this screen.



This screen functions in an identical manner to the **Sample Editor**. You can also access this screen by clicking Define Controls in the

Normalisation

Normalisation of the EndPoint data brings all samples on all channels analysed, within the range of 0-100. You must have selected at least one **Positive Control** and one **Negative Control**, however, you will need more than this if you are analysing multiple channels and your standards are not multi-plexed. You should also use more than one control of each type if there is the risk that your positive control may not react.

1. For each channel, all the Positive Controls are sampled and one with the highest fluorescence is set to be 100%. This allows for a Positive Control to fail without affecting your experiment, if you are running duplicate controls.
2. All the Negative Controls are then sampled, and the one with the lowest fluorescence level is set to 0%.
3. The raw fluorescence values of the other samples are then scaled relative to the highest Positive Control and the lowest Negative Control.

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Here is an example:

Sample	Type	Fluorescence
1	Positive Control	56.3
2	Positive Control	53.0
3	Negative Control	4.5
4	Negative Control	4.3
5	Sample	48.1
6	Sample	6.4

This experiment was a success, as the two positive and negative controls are both close together, and are outside the fluorescence values of the samples 5 and 6.

Here are the normalised values:

Sample	Type	Expression(%)
1	Positive Control	100.0
2	Positive Control	93.7
3	Negative Control	0.4
4	Negative Control	0.0
5	Sample	84.2
6	Sample	4.0

As Sample 1 was the Positive Control with the highest fluorescence, it was set to 100% expression, with the other positive control slightly lower. Sample 4, the lowest Negative Control was set to 0% expression. It is now obvious that Sample 5 is more likely to have reacted, whereas Sample 6 is likely not to have reacted.

NOTE: If Positive and Negative Controls are not selected carefully, it is possible to achieve expression levels of greater than 100%, and lower than 0%. This can be interpreted as "the sample expressed itself more than the positive controls", or "it is less likely that the sample reacted than that the negative controls reacted". Such situations are, however, unlikely to cause errors.

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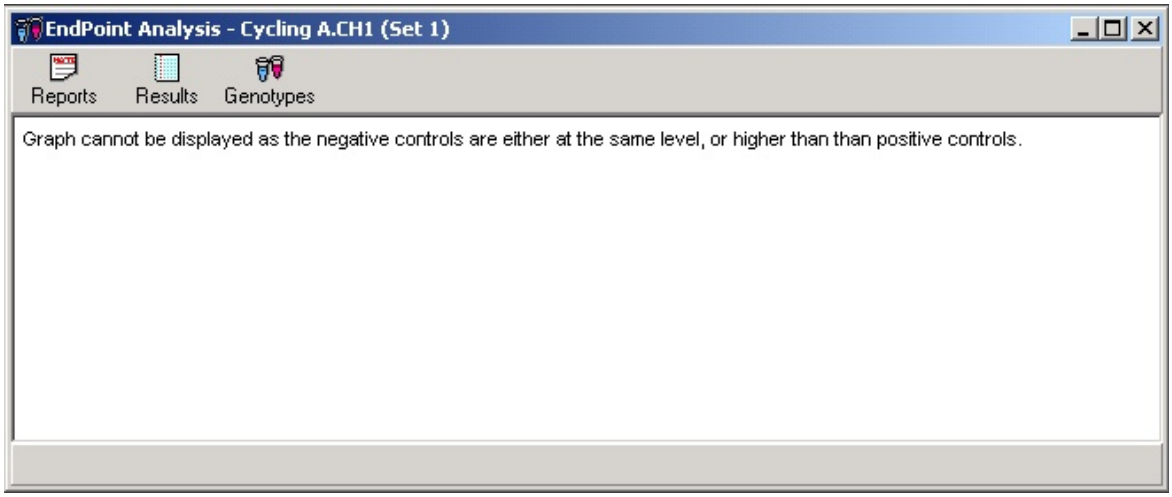
If you receive a message stating that the Negative Controls are higher than the Positive Controls, then you have incorrectly set up your samples. For example, your Negative Controls had a level of 10FI, but the Positive Controls had a level of 5FI. Since there is no way to interpret this in a logical manner, you will receive the following warning:


EndPoint Analysis - Cycling A.CH1 (Set 1)
Reports
Results
Genotypes
Graph cannot be displayed as the negative controls are either at the same level, or higher than than positive controls.

Normalisation on Multiple Channels

If a sample is a positive control for at least one channel, mark its type as Positive Control. Otherwise leave it as type Sample. The same applies for Negative Controls.

If you are analysing data on multiple channels, then you still only define one page of negative and positive controls. So, if a sample is a Positive Control on FAM/Sybr, but not on JOE, you should still define it as a Positive Control. So long as you have another Positive Control on JOE which will react, the control for FAM/Sybr will be ignored. The only limitation of this method of describing the samples is that a single sample cannot be a Positive Control on FAM/Sybr, and a Negative Control on JOE, for example.

Threshold

The **Threshold** is used to determine the percentage level of expression required for a reaction on each channel. Once the **Positive** and **Negative Controls** have been defined, all channels will be normalised to the same scale, 0-100%. This is the reason why only one threshold is needed, even when analysing multiple channels.

Click and drag the threshold line to an area between 0-100%. Your threshold should not be too near samples on either side of the line -- this indicates that the experiment was not conclusive. If the difference between a sample reacting or not reacting is a matter of a couple of percent, then the same reaction run again could appear on the other side of the line.

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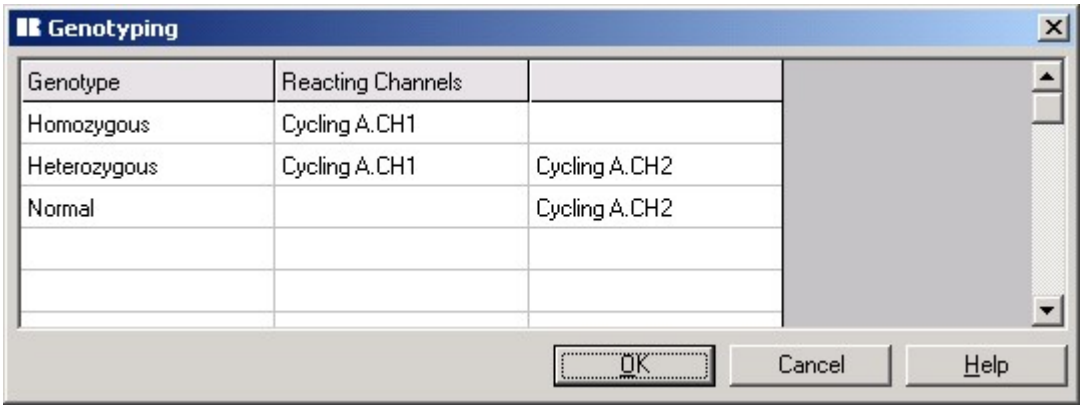
Genotypes

Genotypes: As in Allelic Discrimination, this option opens the genotype window to define which genotype is detected in which channel.



This screen lets you assign genotypes to reacting channels. In the above example, a sample is heterozygous if readings in channels Cycling A.CH1 and Cycling A.CH2 cross the threshold.

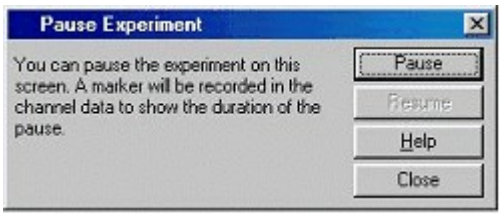
5.7 Run Menu

5.7.1 Start Run

Starts the defined Temperature Profile with the current Gain settings. Before the Rotor-Gene starts the Profile Run Confirmation screen is shown. A graphical representation of the temperature profile to be run is displayed along with the Gain settings for each channel.

5.7.2 Pause Run

You can use this screen to pause and resume an experiment. As performing this operation can seriously affect the results of an experiment, a marker is drawn across all channels acquired to by the currently running cycle. A message is also placed in the message screen.


Pause Experiment
You can pause the experiment on this screen. A marker will be recorded in the channel data to show the duration of the pause.
Pause
Resume
Help
Close

5.7.3 Stop Run

When stopping a run you will be prompted to confirm the end of the run.

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5.8 View Menu

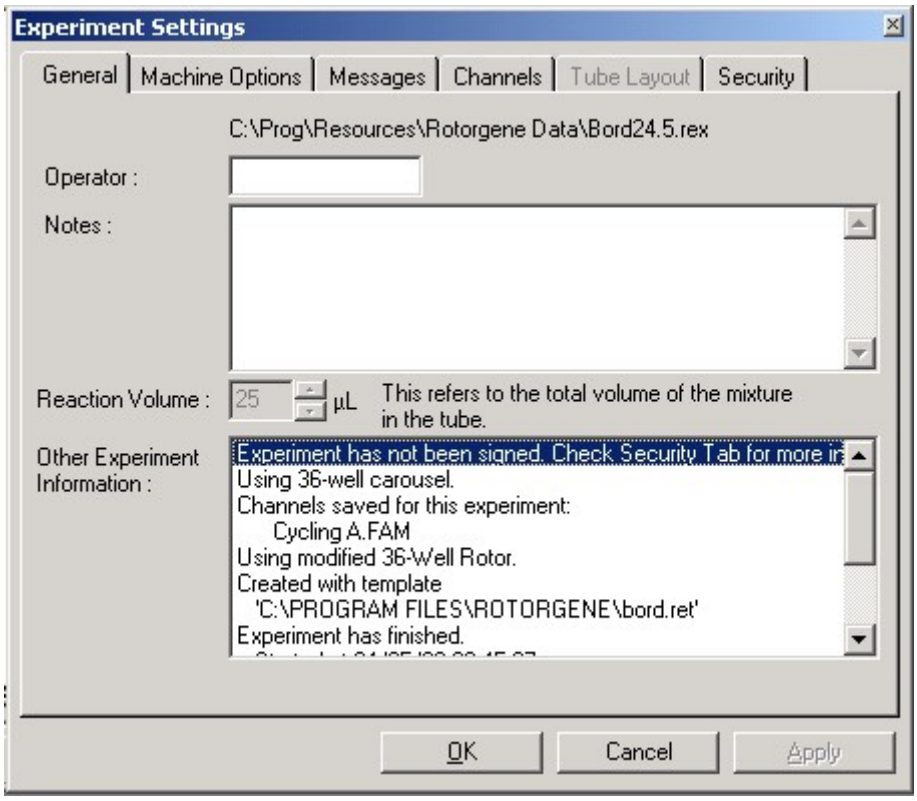
5.8.1 Experiment Settings

General

This window allows the setup of experiment information, experiment name, analysis date, operator anc any associated notes.

The experiment information window contains all information except for the profile required to configure an experiment. The following information is displayed after a run has finished: records the type of machine, Gain settings, number of channels and time of start/finish.

If you were a previous Rotor-Gene user, this screen now contains elements, which were found in separate windows via the View menu.



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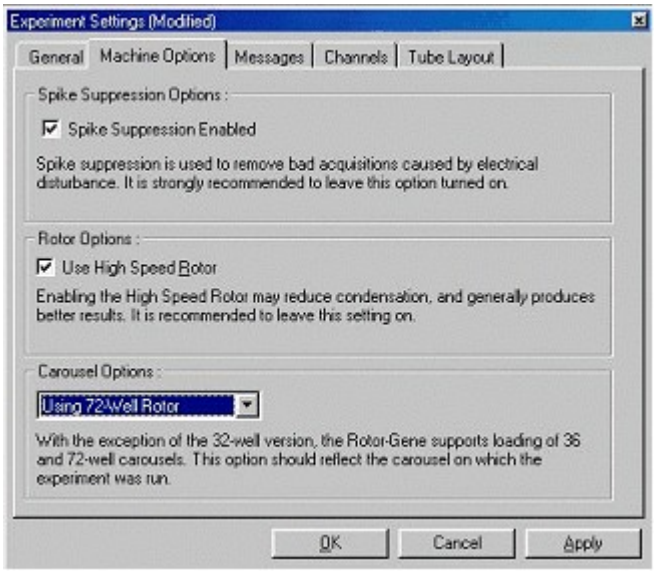
Machine Options

This tab contains settings for the configuration of the Rotor-Gene machine.


Experiment Settings (Modified)
General
Machine Options
Messages
Channels
Tube Layout
Spike Suppression Options :
Spike Suppression Enabled
Spike suppression is used to remove bad acquisitions caused by electrical disturbance. It is strongly recommended to leave this option turned on.
Rotor Options :
Use High Speed Rotor
Enabling the High Speed Rotor may reduce condensation, and generally produces better results. It is recommended to leave this setting on.
Carousel Options :
Using 72-Well Rotor
With the exception of the 32-well version, the Rotor-Gene supports loading of 36 and 72-well carousels. This option should reflect the carousel on which the experiment was run.
OK
Cancel
Apply

Spike Suppression Options: Spike suppression is used to remove spikes caused by electrical disturbance. It is strongly recommended to leave this option on.

Use High-Speed Rotor: The High-Speed Rotor option will spin the rotor more quickly when the machine is cooling. This option is useful as the high speed firms the tubes in their places and prevents them from moving around. This option should generally be left on.

Carousel Options: The machine carousel size should be set to the size of the carousel currently installed in your Rotor-Gene. If opening an existing experiment, this setting will reflect the rotor that was installed in the machine at that time. The options available depend on your machine and the carousels it supports.

Messages

This tab displays messages where the operator has changed functions such as pausing the machine or skipping cycles during a run. It also displays warnings received during the experiment. You should check this tab if you receive unusual results.

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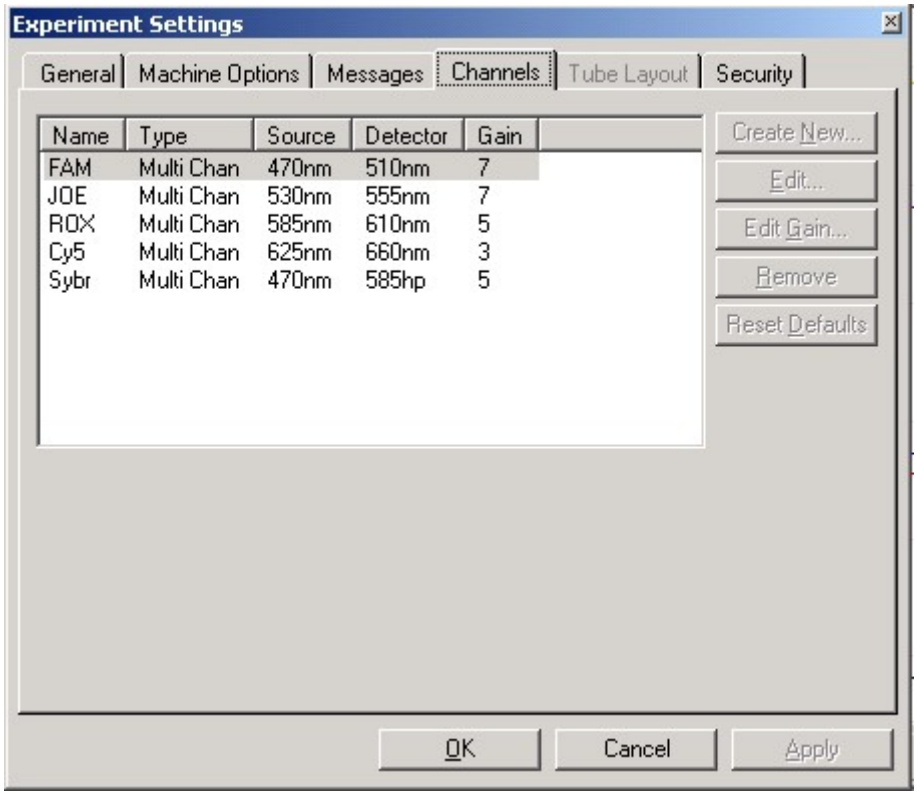
Channels

If configuring a new experiment, the channels tab displays the current configuration of the channels available in the machine. If you are viewing an existing experiment, then the information displayed represents the configuration of the channels when the experiment was run. You can modify the Gain and create and modify channels if you are using a Multi-Channel system. To restore default channels if an experiment corrupts your machine's channel settings, click Reset Defaults.



Name: Describes the name of the channels.

Type: Describes the type of machine chosen (Dual-Channel/Multi Channel). This should be set to Multi-Channel, as on a Dual-Channel filters cannot be changed.

Source: Specifies the wavelength of the source LED.

Detector: Specifies the wavelength of the detection filter.

Gain: Specifies the Gain for that particular channel.

Create new: This feature allows creating new channels. Pressing this button opens a window, which asks for a new name, source and detection filter. The filters can be chosen by using the pull down menu next to each window.

Examples for valid Channel Configurations are:

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Channel No	Source/Detector	Fluorophore
1	**470nm/510nm**	**FAM**, Sybr Green I
2	**530nm/555nm**	**JOE**, VIC
3	**585nm/610nm**	**ROX,** Tamra
4	**625nm/665nm**	**Cy5**
5	**470nm/585hp**	**Sybr Green I** (~2x sensitivity)
6	**470nm/610hp**	**FRET**, Cy5, LC640

Channels 1 to 5 are the standard configuration for 4 channel multiplex detection. Quencher molecules need to be used on the 3' end of the dual labeled probes so as not to occupy spectral bandwidth. The best combination is BHQ (black hole quenchers from Biosearch Technologies) FAM/BHQ1, JOE/BHQ1, ROX/BHQ2, Cy5/BHQ3.

Channel 5 can be used for single channel Sybr Green I detection and gives more sensitivity than Channel 1 due to the fact that a 585hp (high pass filter is used) and so more energy is captured.

Channel 6 is used to detect eg. Cy5 or LC640 in a FRET reaction where an increase in energy is detected. This could be used for quantitation or mutation detection.

NOTE: For mutation detection using FRET probes, the information is contained in the differential of the melt curve and it makes no difference whether the increase in Cy5 or the decrease in FAM is monitored. This is helpful when looking at multiplexing a mutation detection assay as a FAM/BHQ1 and JOE/BHQ1 probe sets can be used in conjunction with Channel 1 and 2 configuration.

Tube Layout

Tube Layout: If you are using a 72-well system, you may wish to reorganize samples to more closely match the labeling that you are using on a 9x8 block. The tube layout tab allows you to label samples sequentially (the default), so that samples are listed in the order they are placed in the machine, or by every 9th sample (1A, 1B, 1C, etc). This is useful when loading samples with a multi-channel pipette.

If modifying this setting, you should perform a test run to confirm that the organization option you have selected works in the manner that you expect.

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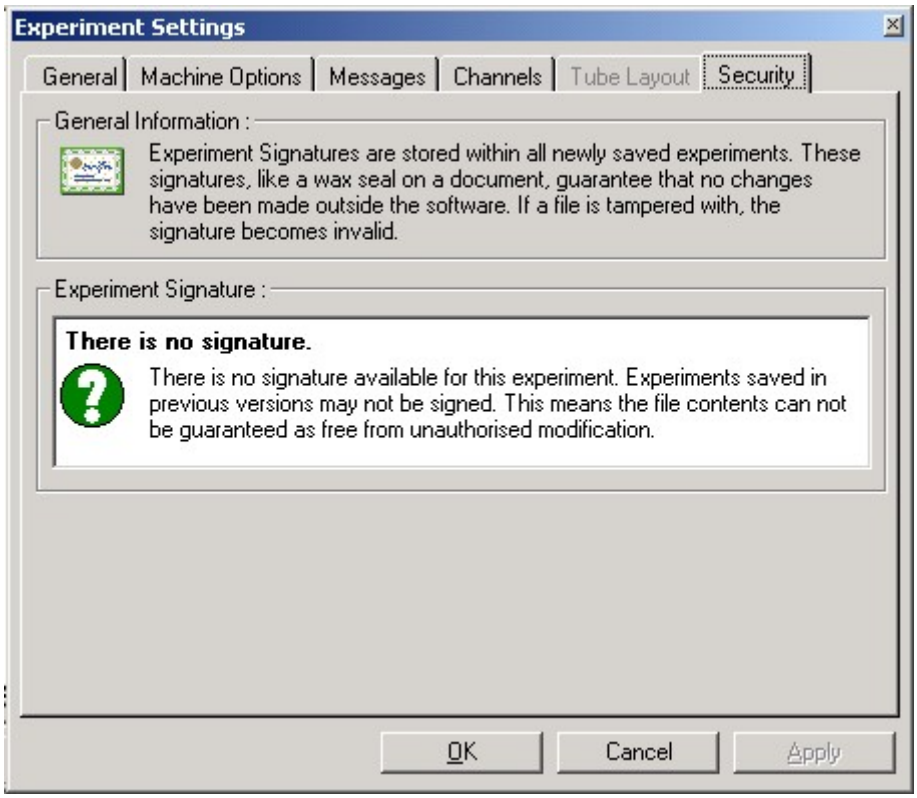
Security

The security tab displays information about the Experiment Signature. The experiment signature is a non-reversible key which is regenerated every time the file is changed. If any section of the REX file is modified outside of the software, the signature and the file will no longer match. Using the signature, you can ensure that a scientist does not modify the raw data outside the application, that the profile has not been tampered with and that the temperature graph is valid. The signature also protects against non-malicious corruption, such as file-system errors.


Experiment Settings
General
Machine Options
Messages
Channels
Tube Layout
Security
General Information :
Experiment Signatures are stored within all newly saved experiments. These signatures, like a wax seal on a document, guarantee that no changes have been made outside the software. If a file is tampered with, the signature becomes invalid.
Experiment Signature :
There is no signature.
There is no signature available for this experiment. Experiments saved in previous versions may not be signed. This means the file contents can not be guaranteed as free from unauthorised modification.
OK
Cancel
Apply

Important: The experiment signature only exists in recent versions of the software. The absence of a signature in old files does not mean that a file has been modified. However, any new experiments you create should contain a signature and so can be validated. You should treat with suspicion any file where the signature is invalid.

You can ensure security for your files by making a laboratory policy that all experiment files must be run using 5.0.8 or later. Experiments run in this version will display a warning if they have not been signed, whereas older experiments will not. However, all reports will include the version number of the software, so you can also treat as suspicious newly run files on old versions of the software.

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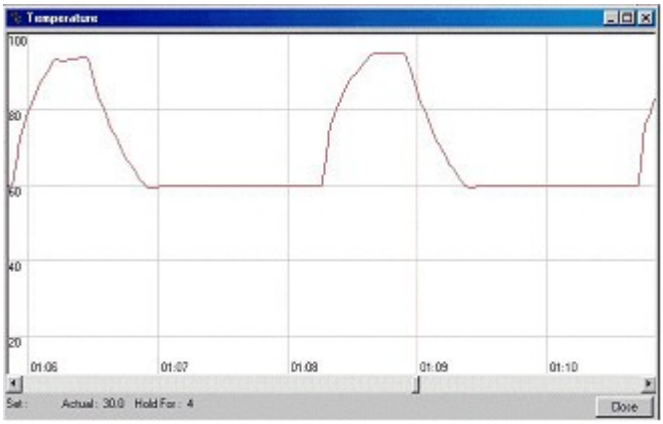
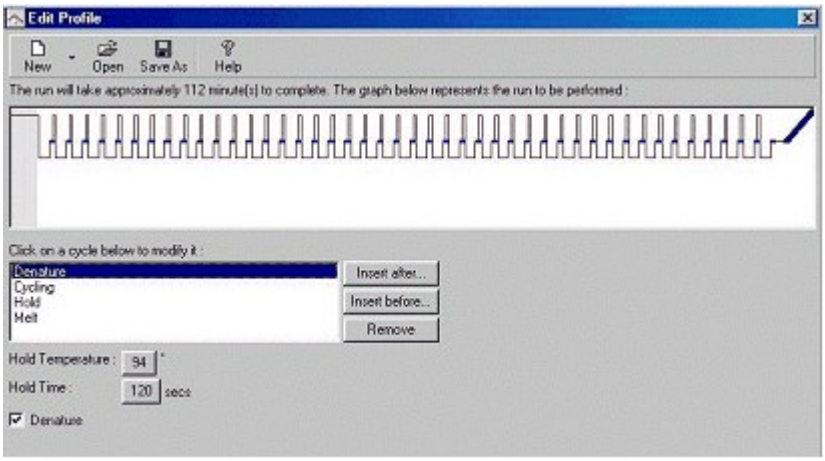
5.8.2 Temperature Graph

The temperature graph shows the temperature of the samples during a run. As the run proceeds the Set, Actual and Hold time is shown for each step of the program. When loading an existing experiment file the temperature graph shows the temperature history during the experiment. The vertical scale is 20-100°C and the horizontal scale is shown in minutes. Use the scrollbar to scroll backwards and forwards throughout the temperature history.



5.8.3 Profile Editor

This screen allows you to preview and modify the profile the machine will follow to heat, cool and acquire data from the tubes in the Rotor-Gene. A profile is made up of cycles, which are listed in order of running in the list box.



Add five cycles (when visible): When cycling has already begun, it cannot be modified. However, it can be lengthened by clicking on this button, enabling you to prolong an experiment. The add 5 cycles button is only visible during cycling. Five cycles could also added in the Profile Progress Screen.

Insert After: Displays a pop-up menu where you can select the type of cycle to insert into the profile. The new cycle will be inserted after the currently selected cycle.

Insert Before: Identical to the Insert After button, except that the new cycle will be inserted just before the currently selected cycle.

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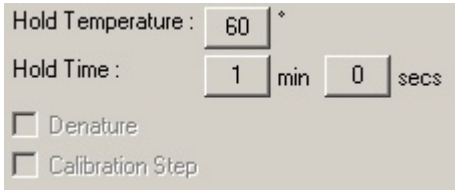
Remove: Removes the currently selected cycle from the profile.

Depending on which cycle is currently selected, the lower half of the screen will change to provide options specific to that cycle type. Each cycle type and its related options are discussed below.

Hold

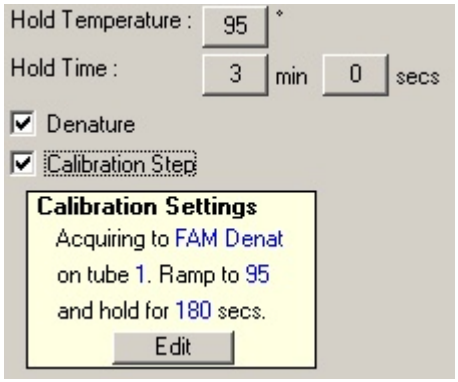
This cycle has a dual purpose. It can either instruct the Rotor-Gene to remain at a temperature for a given amount of time, or it can perform an optical denature calibration.

Click directly on the value you would like to change, such as 60 degrees to bring up a seperate edit window. This window will allow you to modify the value using either a slider control, or by entering it directly into the textbox.



Denature: To perform an optical denature calibration, the hold temperature must be 90 degrees or above. After changing this, the Denature option becomes available. If you are using **Optical Denature**, you should also tick "Calibration Step" to allow the software to locate the melt point of the samples during this hold.

The calibration settings seen below are the same as those described in the section Adding a New Optical Denature Cycling.



If **Calibration Step** is not selected, ticking the Denature box has no effect other than changing the name of the cycle.

Optical Denature Cycling

What is Optical Denature Cycling?

Optical Denature Cycling is an exciting new technique which performs real-time melt analysis to determine the melt peak of a reference sample. This indicates with greater precision when your product has denatured than by setting a particular denature temperature for a hold time. To perform this technique, simply place a tube of pre-amplified product in tube position 1 in your rotor. The reference tube must also contain a detection chemistry that enables determination of the dissociation

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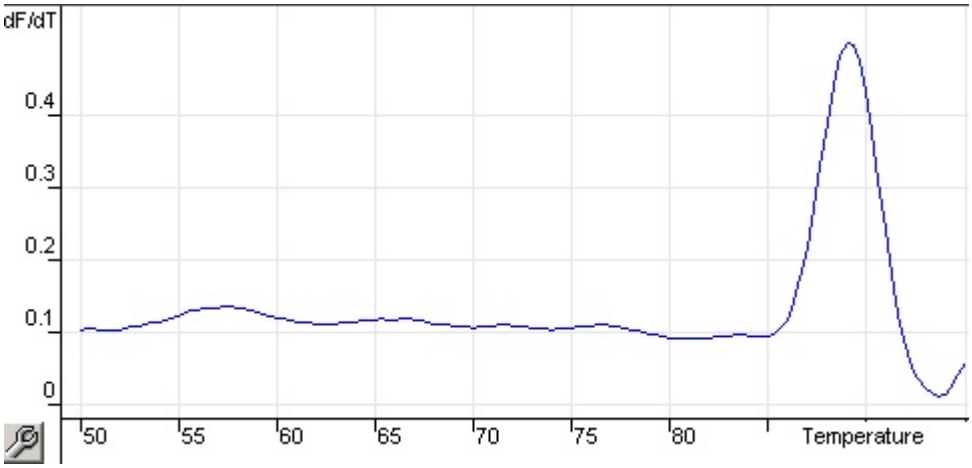
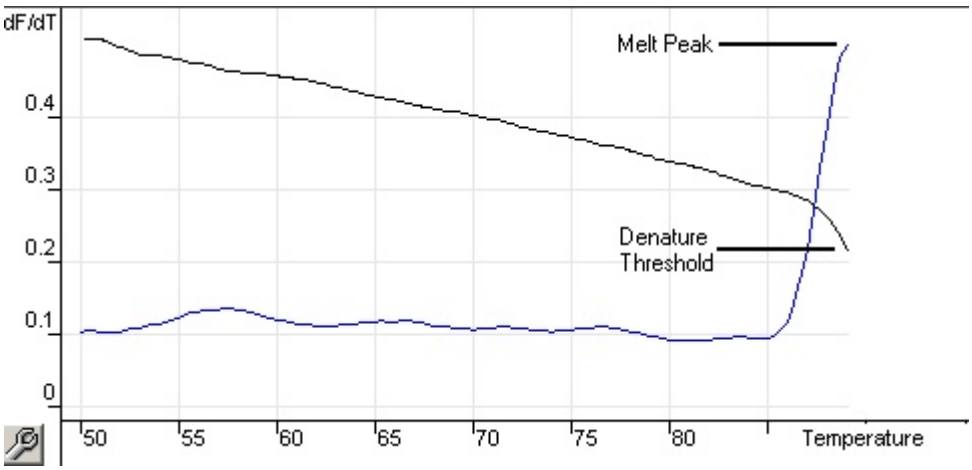
of the strands of the product. We suggest using Sybr Green I.

When first heating the machine to the initial denature temperature, a melt on FAM/Sybr is performed starting from around 80 degrees until 95 degrees (although these parameters may vary). From this data, a melt curve is generated and automatically analysed:



The **Melt Peak** is referenced back to the raw data to obtain a **Denature Threshold**. Then, every Optical Denature step, the machine is heated as quickly as possible and data acquired continuously. Once the **Reference Tube** has hit this fluorescence level, the machine is immediately cooled and move onto the anneal step. While cycling, a peak is not calculated, rather, it is the fluorescence level that is referenced to the melt peak and designates the denature threshold.

In this graph, the raw fluorescence readings and the first derivative have been overlayed. It shows the 1-to-1 correspondence between the **Denature Threshold** and the **Melt Peak** obtained during the calibration.



What do I need to perform an Optical Denature run?

To perform an Optical Denature run, you will need:

- A pre-amplified sample which you should place in position 1 in the rotor. This sample should contain the same product as samples of interest and a detection chemistry, such as Sybr Green, that allows for the monitoring of product dissociation.

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- An optical denature profile. You can find out how to set up an Optical Denature Run in the topic Adding a New Optical Denature Cycling.

What will I see when performing an Optical Denature run?

An Optical Denature run, from the user perspective, appears almost identical. The most striking differences are the melt step automatically inserted at the beginning of the profile, and the sharp profile of the denature step during cycling. The denature step does not require defined hold times as the dissociation of the product is monitored at each cycle.

Configuration

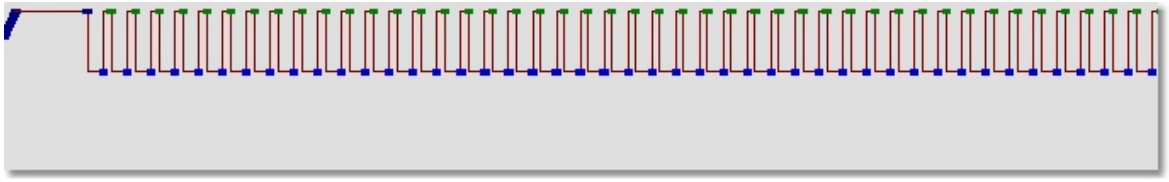
To perform this technique, the software must know several things about your experiment:

- The Initial Denaturation temperature. This is the same temperature as your denature step in a standard cycling profile.
- The tube position of a sample which has already been amplified, and which will produce a melt curve on the FAM/Sybr Channel. The tube position of a sample which has already been amplified, and which will produce a melt curve on the FAM/Sybr Channel. For example, if you are performing a Sybr-Green I run or a run with dual labeled probes, put a sample of pre-amplified Sybr product in tube position 1, and your other samples in the remaining positions.
- The time of denaturation required. The time depends on the used Taq-Polymerase and can vary between 1 and 15 minutes.

You must also define an Optical Denature cycling profile. This is enabled by default in this version of the software. However, your existing templates from older versions of the software will not use this step.

Adding a New Optical Denature Cycling

To add an optical denature step, open the Profile Editor. Then click New. A default profile containing a Denature step and an Optical Denature Cycling step will appear: The profile is displayed as:



The blue shaded region at the beginning of the run represents the optical denature calibration process. The Green dots represent the acquisitions taken each cycle during heating. The lighter blue dots represent the acquisition at the end of the anneal step at 60 degrees. Note that while the profile shows each step going to the same denature temperature, this may not be the case. If the sample requires slightly longer to melt towards the end of the run, the optical denature process will wait for the melt in the fluorescent data, and not off the times. For this reason, the temperature trace for optical denature may vary for each cycle.

By clicking on the Cycling step, the information about the 2-step profile appears in the lower half of the Profile Editor window. Click on the second half of the graph with the Optical Denature symbol .

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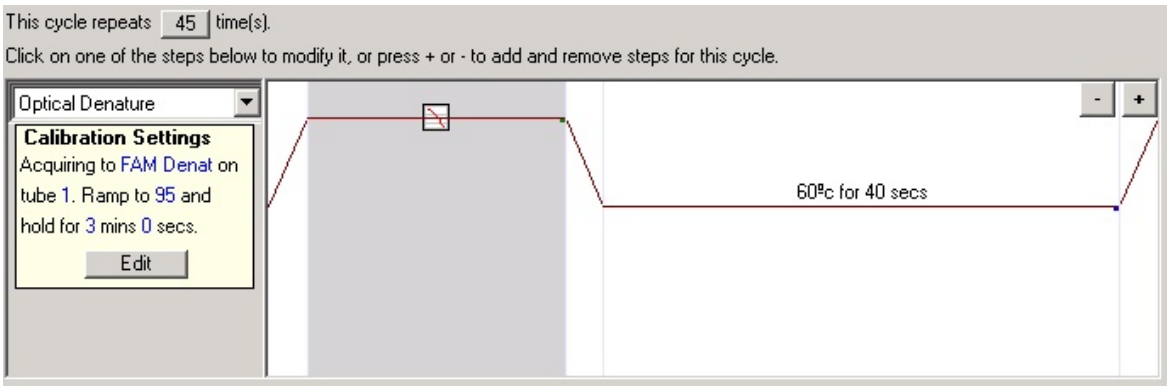
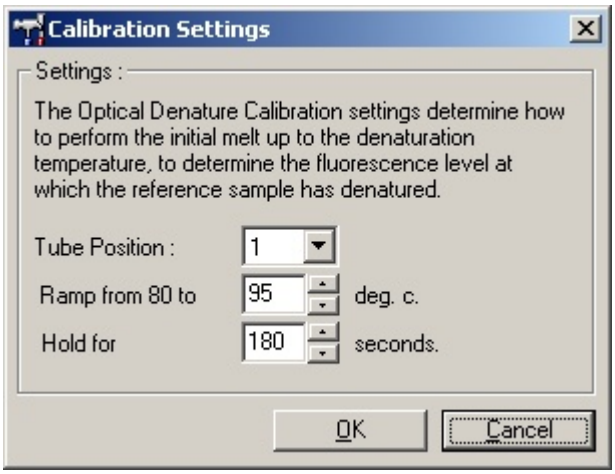
The Calibration settings information appears on the left-hand side of the screen:


This cycle repeats 45 time(s).
Click on one of the steps below to modify it, or press + or - to add and remove steps for this cycle.
Optical Denature
Calibration Settings
Acquiring to FAM Denat on tube 1. Ramp to 95 and hold for 3 mins 0 secs.
Edit
-
+
60ºc for 40 secs

Most of the time, this information will be correct. You can, however, modify it by clicking **Edit**. The Calibration Settings window will then be displayed:


Calibration Settings
Settings :
The Optical Denature Calibration settings determine how to perform the initial melt up to the denaturation temperature, to determine the fluorescence level at which the reference sample has denatured.
Tube Position : 1
Ramp from 80 to 95 deg. c.
Hold for 180 seconds.
OK
Cancel

You should ensure that:

- The tube indicated in the Tube Position contains a sample of pre-amplified product that will show a melt peak on the FAM/Sybr channel.
- The Final Ramp temperature will not burn the sample, yet will be high enough to allow it to melt.
- The hold time is sufficient to denature the samples.

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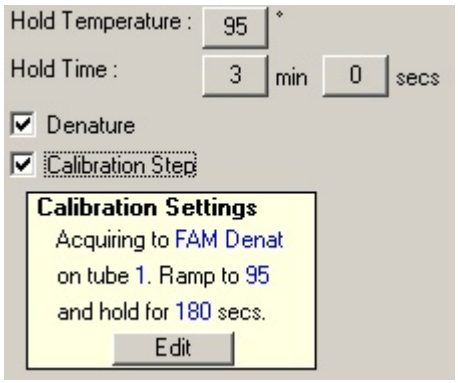
You can also define a Denature step yourself, and configure the calibration settings via its screen:



The calibration settings are sychronised with the denature settings, so a change to the hold time in the denature step will automatically update the calibration's hold time. This is because the calibration process and denaturation are equivalent in **Optical Denature Cycling**.

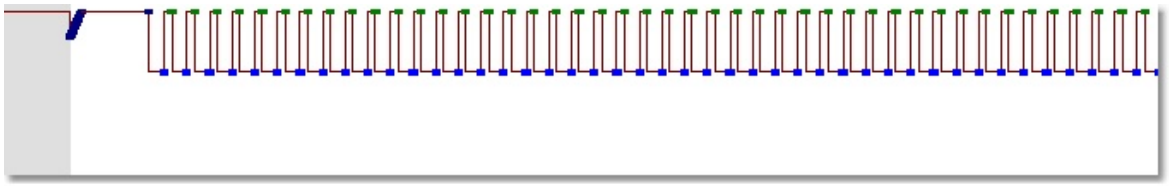
Changing An Existing Step to Use Optical Denature

To change an existing denature step in a Cycling, select the cycle in the Profile Editor's list. Then, select the denature hold by clicking on it in the preview graph at the bottom of the screen:

Click on the word "Timed Step" and select "Optical Denature". The Temperature and Hold Time will be removed and the Optical Denature icon will be displayed in their place.

IMPORTANT NOTE:

- If you are using Optical Denature Cycling, you **must** make sure that your denature step is also a calibration step. Otherwise, the Cycling step will automatically include one as well. The profile will appear with a dip in the denature, as shown below:



To correct this problem, tick the **Calibration Step** box in the Denature step.

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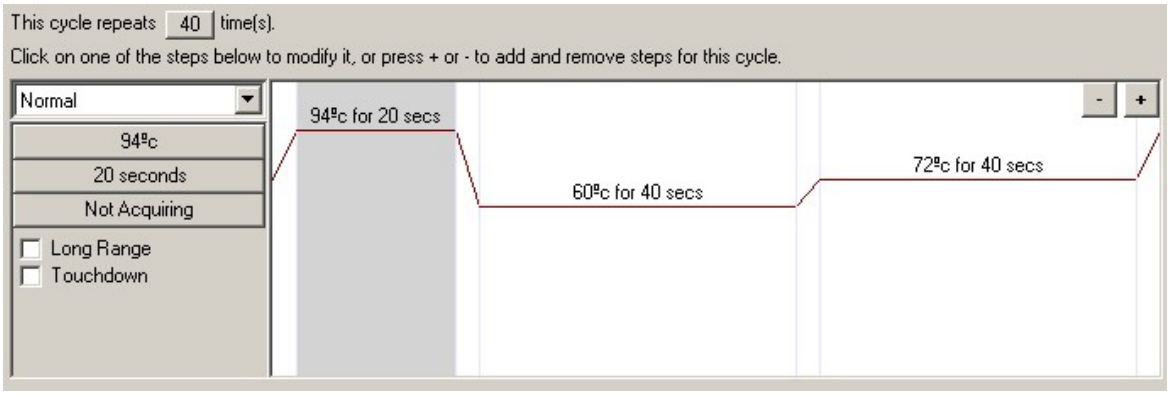
Cycling

As its name implies, Cycling loops through the given cycle points a given number of times, acquiring at certain points, even gradually cooling or lengthening certain cycle points if desired as it repeats.

The white graph represents a single repeat in a cycling profile. More cycle points can be added to the end, or the tail cycle point removed by using the [+] and [-] buttons. This gives you the flexibility to define just one cycle point (in which case you can acquire at a constant temperature), or with up to five points.



To modify the temperature, hold time or acquisition properties of a cycle point, first click on that portion of the graph. The graph will become greyed-in, such as the 94 degree step shown here. Then, click on the value you are interested in in the left-hand bar. For example, to modify the temperature, click on 72 degrees to display a pop-up window where you can modify this value. The operation is identical for hold time. You can also drag the graph line up and down to modify temperature, and from left to right to modify the hold time.

Touchdown: Touchdown can be enabled to decrease the temperature during the initial cycles. Set the number of cycles and the program decreases the Anneal Temperature by 1°C every cycle for the number of cycles specified. This is reflected in the graphical cycle representation.

Long Range: Long range can be enabled to increment the hold time of the selected step with each new cycle. This has the effect of increasing the extension time as the cycling proceeds.

Acquisition

For acquisition, a separate window will appear in which you can modify which channels will be acquired to:

Use the [<], [>] and [<<] buttons to move channels between the two lists displayed. All channels you

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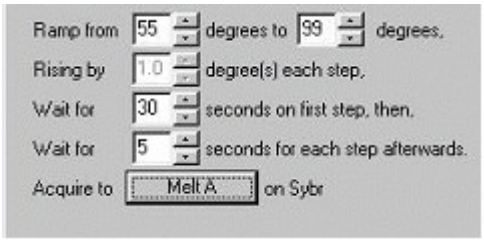
put in the right-hand side will be acquired to. To not acquire on this cycle point, click on **Don't Acquire**. Any changes you make will be reflected back on the Profile Edit window.

Melt

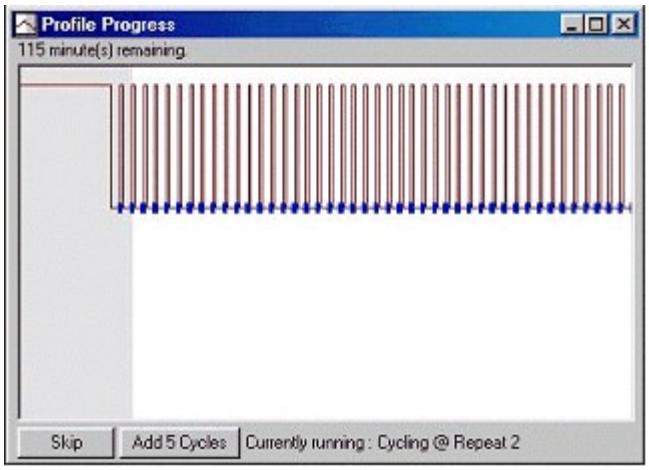
For a melt cycle, specify a start temperature, an end temperature, the time you want to wait at before the first acquiring point, the amount of time to remain at each point and a ramp will be generated going between the two temperatures. The **Acquiring To** option, here set to **Melt A**, can be changed by clicking on the button. The same screen as for Cycling will appear and the channels to acquire to can be selected.


Ramp from 55 degrees to 99 degrees.
Rising by 1.0 degree(s) each step.
Wait for 30 seconds on first step, then.
Wait for 5 seconds for each step afterwards.
Acquire to Melt A on Sybr

When running a standard melt the temperature is increased by increments of 1ºC.

5.8.4 Profile Progress

This screen shows a graphical representation of the thermal profile associated with the experiment. When running an experiment the shaded portion of the window indicates the number of cycles that have been completed. There is also an estimate of how many minutes the program will take to finish this run.



There are two further buttons on the buttons, **Skip** and **Add 5 Cycles**. While skip allows skipping any steps of the profile, the "add 5 cycles" button allows to add five cycles to the cycling profile.

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5.8.5 Edit Samples

This window has identical functionality to the sample edit grid in the New Experiment Wizard, except that the toolbar functions are also available in the File and Edit menus. This menu can also be accessed by right clicking over the sample list on the right side of the main screen.

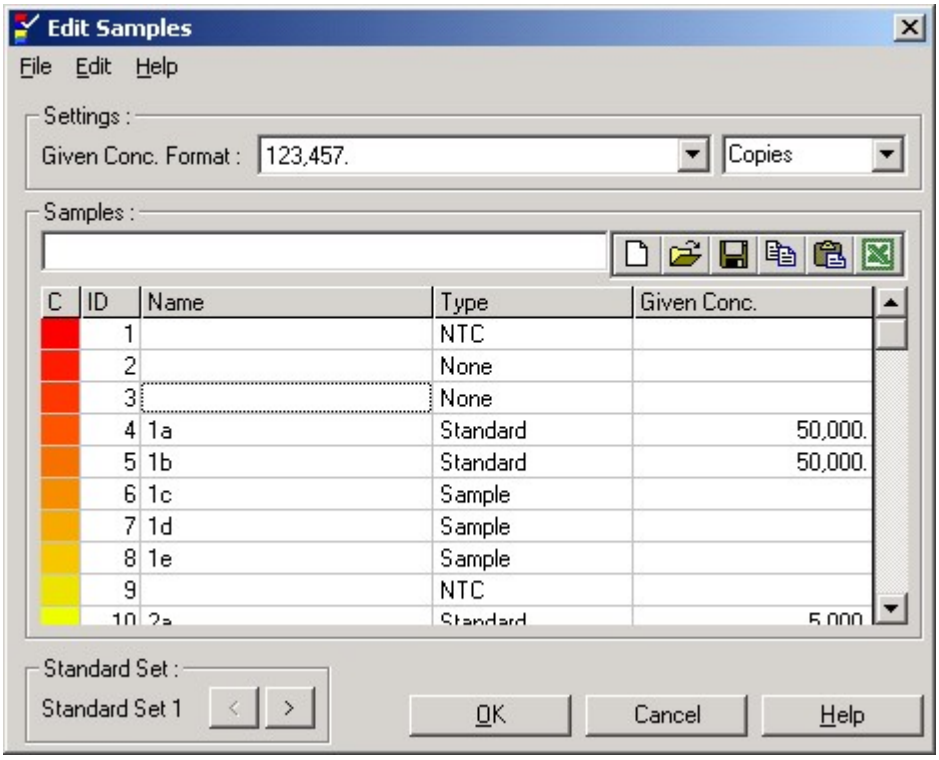
Three menus are given on the top of the screen, **File**, **Edit** and **Help**. The file menu is used to create a new (blank) sample sheet, open an existing sample template or save sample names as a template for future use. The extension of those files is *.smp

Given concentration format: This drop down menu is used to choose a concentration suitable format. Due to the fact that different countries show formats in different ways, various formats are given to choose from.

Unit: This drop down menu allows choosing an appropriate unit for your assay.

Edit: Pressing this button opens the color scheme. Selected samples (up to 36 or 72) will be changed according to the color chosen. Click OK and all the selected samples will be assigned this color.

Reset Default: Click this to reset all selected color cells back to their default color values.



Gradient: The gradient function allows choosing a gradient form the first to the last selected color. A gradient has been used in the above sample list. Several gradients can be chosen in a sample setup.

Export Samples: To export data into an Excel spreadsheet simply use the right mouse button. Excel will automatically be opened and exports the samples. Using the **Export Samples...** function in the main window will also create a file that can be can exported into a spreadsheet application. Importing samples from Excel into the Rotor-Gene software is also possible by simply using the copy and paste

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function.

Tab Key: The Tab key can be used to navigate around the sample editor.

New Icon: Clears the sample grid in preparation for data entry.

Open Icon: Brings up a dialog box in which you can select a Rotor-Gene Sample file to import. Note: A 32-well sample setup cannot be imported into a 72-well setup and vice versa. The number of samples in the open sheet and the file being imported must match.

Save As Icon: Brings up a dialog box in which you can enter the name of a file to save the current samples to.

Copy Icon: Copies the selected cells.

Paste Icon: Pastes cells which had been selected with the copy command onto the currently selected position on the grid.

Excel Icon: Pressing the Excel icon prompts you for a file name. Excel is than opened automatically.

Sample Types: There are several sample types listed.

Sample Type	Meaning
None	No sample in that position.
NTC	No Template Control
-ve Control	Negative Control
+ve Control	Positive Control
Sample	Unknown sample to be quantitated or melted.
Standard	Values are used to construct a standard curve to calculate unknown sample concentrations.

Standard Sets: This new function allows the user to choose various standard curves in the same run. Use the arrow buttons to move from one standard set the next. The software only allows you to move further if standards in the previous standard set have been defined.
This feature is very useful when different standard curves need to be run, either in one channel (for example Sybr-Green I or any other fluorphore) or in different channels (with up to four different fluorphores).

Given Conc.: Shows the concentration for each of the standards defined. The units can be defined as a decimal or log number. If a dilution series has to be typed in it is only necessary to type the first two standards. By pressing ENTER, the program automatically adds the 10 fold concentration, if there are further Standards defined below.

Line Style: You can modify the style of the line to improve readability of graphs on black and white printers. You can also add emphasis to certain lines by modifying their style. To access this feature, click on the right arrow button next to the Edit button:

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The toolbar will then change to show the default style "Solid". You can change this to "Dashed", "Dotted" or a number of other possibilities. When you have finished, click the left arrow button to return to the Edit, Reset Default and Gradient view.

Productivity Tips:

Autocompletion: When entering standards, if you place them in consecutive tubes, begin entry on the first tube, then press Enter. Enter the second concentration, then press Enter. The software has automatically predicted the next concentration in the set. Keep pressing Enter to fill in the remaining rows.

Multiple-Row Entry: If you need to enter the same information for several rows at once, select all the rows, then begin to type. The information will be entered into each row. This works for selecting sample types, choosing colors or entering concentrations.

Sample Type Hotkey: To quickly select a sample type, just enter the first letter of its name. So, to set 5 samples to be Non Template Controls, select them in the sample type column, then press N for NTC. All samples will be converted to NTC's. NOTE: Since Sample and Standard have the same beginning letter, press S until the one you want is selected.

Note: A complete sample description can be saved as a sample file (*.smp) and loaded into future experiments with the same sample configuration.

5.8.6 Gain Calibration

When setting up a new experiment with reactions that have not previously been run on the Rotor-Gene it is helpful to use the Gain calibration function. This screen allows you to set the Gains of each of the channels and the set temperature.

Note: The gain calibration will never be 100% correct. This could be due to changes in fluorescence after the first denature step. Nevertheless, the result of the Gain calibration will give you a good indication on what fluorescence level the run will be started.

The Gain for each channel is 1 to 10, 1 = least sensitive and 10 = most sensitive.

When running reactions for the first time it is advisable to prepare a test sample containing all reaction components. This is then placed in the machine and Gain Calibration is run to determine the best Gain setting.

Auto-Calibration

This window lets you calibrate your machine by automatically adjusting your Gain settings until the readings for all selected channels fall below a certain threshold. You can select to calibrate all channels, or just those that you will be using in the current experiment. **Note: This is only available on Rotor-Gene 3000, or the 4-channel, Multi-Filter Rotor-Gene 2000.**

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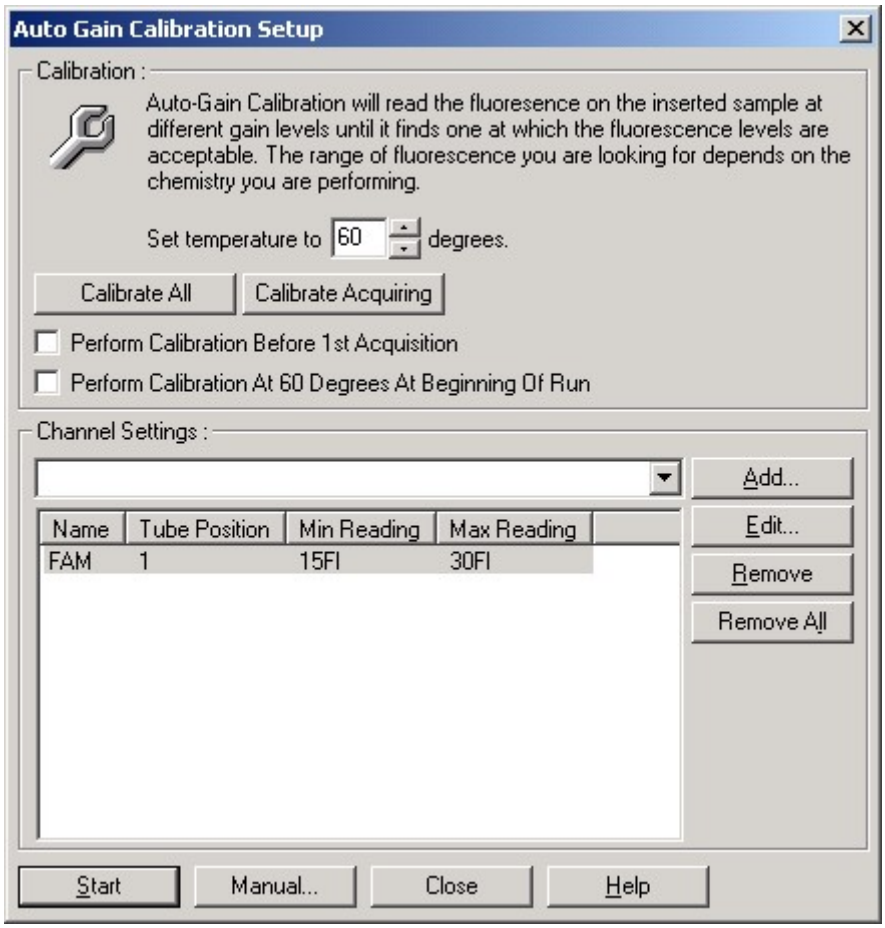
Set temperature to ... Before reading, the machine will be heated or cooled to match the given temperature.



Calibrate All / Calibrate Acquiring: "Calibrate All" will attempt to calibrate for all channels known by the software. Selecting "Calibrate Acquiring" will instead only calibrate those that you have used in the thermal profile defined in the experiment (cycling and melt).

Channel Settings: This is a pull down menu allowing you to add additional channels to the gain calibration window. Choose the channel of interest and **Add**.

Edit... Opens a window where the fluorescence range of the sample can be determined. The auto-calibration process begins on Gain 5, reading from each channel. It chooses the first Gain, which has a fluorescence reading equal to or below the level you set in this box. In the example below, tube position 1 was chosen with a target sample range between 5 and 10 Fl.

Remove and Remove all: Removes the highlighted channel or all channels.

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Start: Begins the calibration process. A Gain will be chosen which is within the given range. If no value is found within the range, the closest match will be chosen.

Manual: Opens the Manual Calibration screen (see below).

Close: Closes this window. You cannot close the calibration window while the machine is in operation.

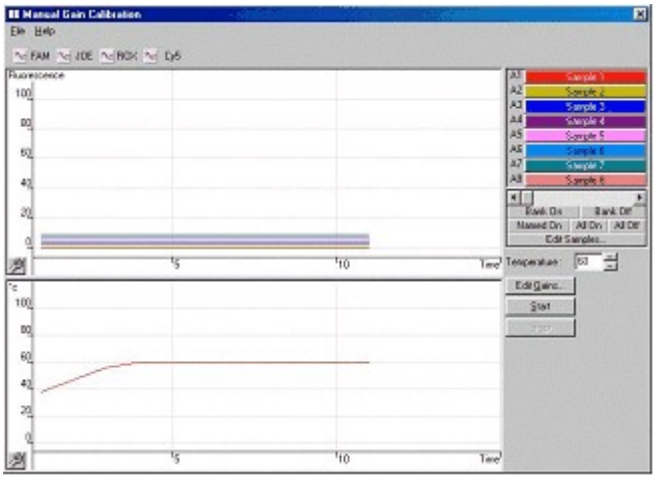
Perform Calibration Before 1st Acquisition: This tick box performs the Gain calibration at the first cycle where data acquisition occurs. This is useful as with some probes after the initial denaturation the background fluorescence of the probes could change substantially.

Perform Calibration At [x] Degrees At Beginning of Run: This tick box performs the Gain calibration just before starting the experiment. The machine is heated to the given temperature, the gain calibration is performed, and then cycling begins on the first step, usually a Denature. This can be useful when a calibration during the run may impact too much on the time spent on the initial step. Usually, however, **Perform Calibration Before 1st Acquisition** should be the ideal option as calibration is performed as close as possible to experiment conditions.

Changing Gain During a Run: If the Gain at the beginning of the run was accidentally chosen to high or to low it can be changed within the first ten cycles. A vertical line in the main screen will appear where the Gain has been changed. Due to the drop or increase in fluorescence the cycles before the change will be excluded from the analysis.

Manual Calibration

This window lets you view in real time the fluorescent readings at any given temperature. It is used when the background of a sample is unknown and therefore the Gain has to be determined to ensure the sample signal is sufficiently on scale.



By default, the first eight samples are toggled on. If more than the first samples would like to be monitored, turn on the samples of interest. Using manual gain calibration means that data are always from all samples.

It takes approximately 4 seconds to acquire data per channel. During this time the user interface is inactivated and so it is best to 1) Start the test 2) wait for the temperature to stabilize 3) note the end point fluorescence (Fl) reading 4) stop the unit 5) make the appropriate Gain change 6) and restart the unit.

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Temperature: Change this value to set the temperature of the machine. Note: The temperature will only be sent when the start button is pressed. Adjust the temperature on the Gain calibration screen to reflect the required acquisition temperature for the run.

Edit Gains: Opens the Edit Gains window. The Gains will be sent the next time the machine is started in the calibrate window.

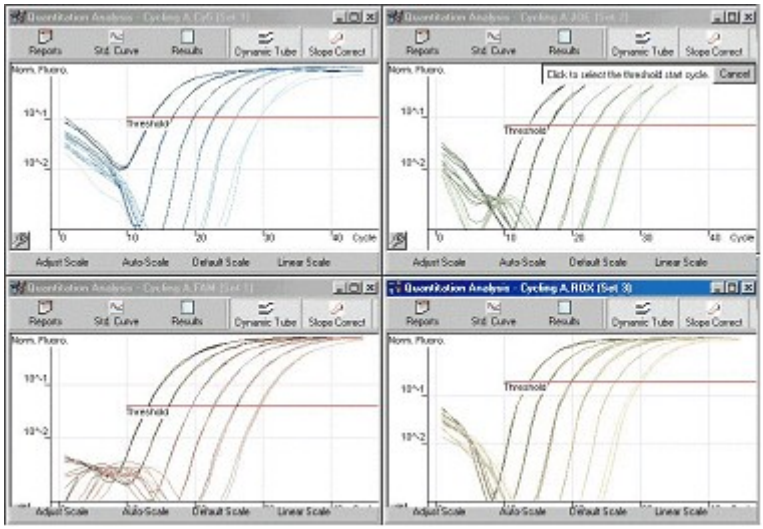
Start: Begins the run, setting the machine temperature to that displayed on the screen. The temperature and channel graphs will start to display data. Ensure the Rotor-Gene displays READY before starting another calibration run.

Stop: Stops the experiment. If the experiment is still acquiring data when you click the button, then the machine will first finish acquiring, and then stop the machine. This process can take up to 5 seconds for each channel data is being acquired.

NOTE: The aim of the Gain setting is to have all data on the screen. The Gain doesn't influence your data. If the Gain was chosen too high, curves might go off scale and data could be lost. It is therefore a good idea to start a Sybr-Green I or dual-labeled probe run with a low fluorescence (an increase is expected) and a quenched FRET run with a higher fluorescence (a decrease is expected).

5.8.7 Display Options

Show at most two analysis windows: This option ticked shows a maximum of two analysis windows at once. By performing a quantitation analysis, three windows are opened by default. If more than six or seven windows are opened at once, the overall view might not be clear. This option ticked will close the first analysis window and replace it with the last opened analysis window. If the option is unticked more than three analysis windows could be displayed. This could be useful when comparing quantitation screens of four different channels.



Show at most 6 windows: To improve readability, the software removes unused windows when new windows are opened. This option is enabled by default, as it keeps the Rotor-Gene work area clear. You may, however, need to see more than 6 windows at once, in which case you should uncheck this option.

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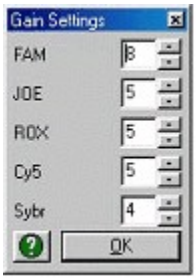
Reset all "Don't Show This Message again" Dialogs: This window forces the software to re-display all dialog boxes again. These include messages about suspicious settings which you may have previously set to not display again. This might be useful if a new user is now using the machine who is unfamiliar with the Rotor-Gene or the Rotor-Gene software.

5.9 Gain Menu

Here, you can view the **Gain Settings** for the current experiment and modify them if the experiment has not yet been run. Use the **Up/Down** arrows next to each text field to modify the fields without needing to enter in a value with the keyboard. Click OK when you have finished.



Sets the Gain of the specified channel before running a program. Gain settings will be retained from the last run.

Change gain during the first ten cycles: This new software version also offers the possibility to change the gain during the initial cycles. A red line will be drawn in the appropriate channel showing where the gain has been changed. The cycles before the gain has been changed will be excluded of the calculation of the quantitation data.

Note: This is only possible for 4-Channel Rotor-Gene, not for dual channel or 32-well Rotor-Genes.

5.10 Window Menu

This menu allows you to tile the window either vertically, horizontally or arrange windows in a cascade. A further option of tiling windows is found under Arrange in the tool menu.

5.11 Help Menu

The **Contents** contains all the help files sorted by names. Simply go to contents and choose the area you are interested in. **What' New** gives a brief overview of new features added to the previous software release. **Context Sensitive Help** will open the help file according to the activated window in the Rotor-Gene software. Pressing **Corbett Research Website** will automatically open our Web page, whereas the forum can be accessed by pressing **Rotor-Gene Online Forum**. **About Rotor-Gene** tells you several details for example the serial number of the Rotor-Gene or the software version you are using.

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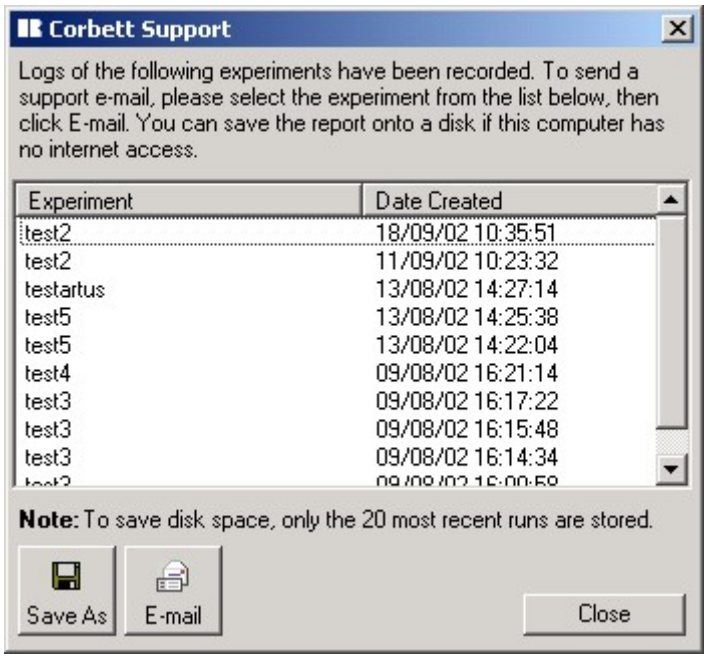
5.11.1 Send Support E-Mail

The **Send Support Email** option in the Help menu allows you to send a support email to Corbett Research which contains all relevant information to an experiment. The **Save As option** will save all the information to a file which you can copy onto a floppy disk or onto a network if you do not have access to email on the Rotor-Gene machine.


Corbett Support
Logs of the following experiments have been recorded. To send a support e-mail, please select the experiment from the list below, then click E-mail. You can save the report onto a disk if this computer has no internet access.
Experiment
Date Created
test2 18/09/02 10:35:51
test2 11/09/02 10:23:32
testartus 13/08/02 14:27:14
test5 13/08/02 14:25:38
test5 13/08/02 14:22:04
test4 09/08/02 16:21:14
test3 09/08/02 16:17:22
test3 09/08/02 16:15:48
test3 09/08/02 16:14:34
Note: To save disk space, only the 20 most recent runs are stored.
Save As
E-mail
Close

6 General Functions Used Over Several Windows

6.1 Opening A Second Experiment

While running an experiment, a second already finished experiment can be opened and analyzed. Note that several functions like **New** or **Start Run** are not activated in this second window. Even closing the first experiment will not bring up those functions. A new run can only be started from the first experiment, once the experiment has finished. If a run has to be started from the second experiment, it needs to be closed and reopened to access the appropriate functions.

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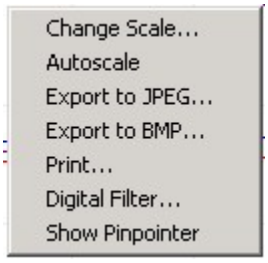
6.2 Spanner Icon

By clicking the spanner icon, you have access to a number of tools, which simplifies manual analysis of data. Those tools could also be accessed using the right mouse button in the appropriate screens.



Scaling: See above

Export to JPEG... Saves the graph in the JPEG image format and **Export to BMP...** saves the graph in the Bitmap format.

Print: This function allows for the current graphic screen to be printed.

Digital Filter: Modifies the currently selected digital filter on the graph. The digital filter smoothes data using a sliding window of points. To open the window, click the right mouse button over a window containing data.

Show Pinpointer: Opens a window with exact coordinates of the current cursor position.

Note: The following options are no longer available via the Spanner icon. You should first drag a selection area on the graph, and then, upon releasing the mouse, a context menu will appear. Choose one of the following options to select samples or zoom the graph.

Select Samples: Shows only the samples that you swipe in a rectangular region.

Zoom: With the zoom function you can zoom in certain areas of the screen. It can be horizontally and vertically.

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6.3 Scaling Options

Adjust Scale will bring up a window in which you can manually enter a scale, or interactively select one. To chose this option simply press the right mouse button over the appropriate screen.

6.4 Abbreviations

BHQ	Black Hole Quencher
Comp. Quantitation	Comparative Quantitation
LCD	Liquid crystal display
LED	Light Emitting Diode
PMP	Photomultiplier
Quant. Settings	Quantitate Settings
REA	Rotor-Gene Archive File
REX	Rotor-Gene Experiment File
RG	Rotor-Gene
SMP	Sample File
Std. Curve	Standard Curve

6.5 Auto-Scale

Auto-Scale attempts to fit the scale to the maximum and minimum readings in the data.

Default: will reset the scale to show from 0-100 fluorescence units.

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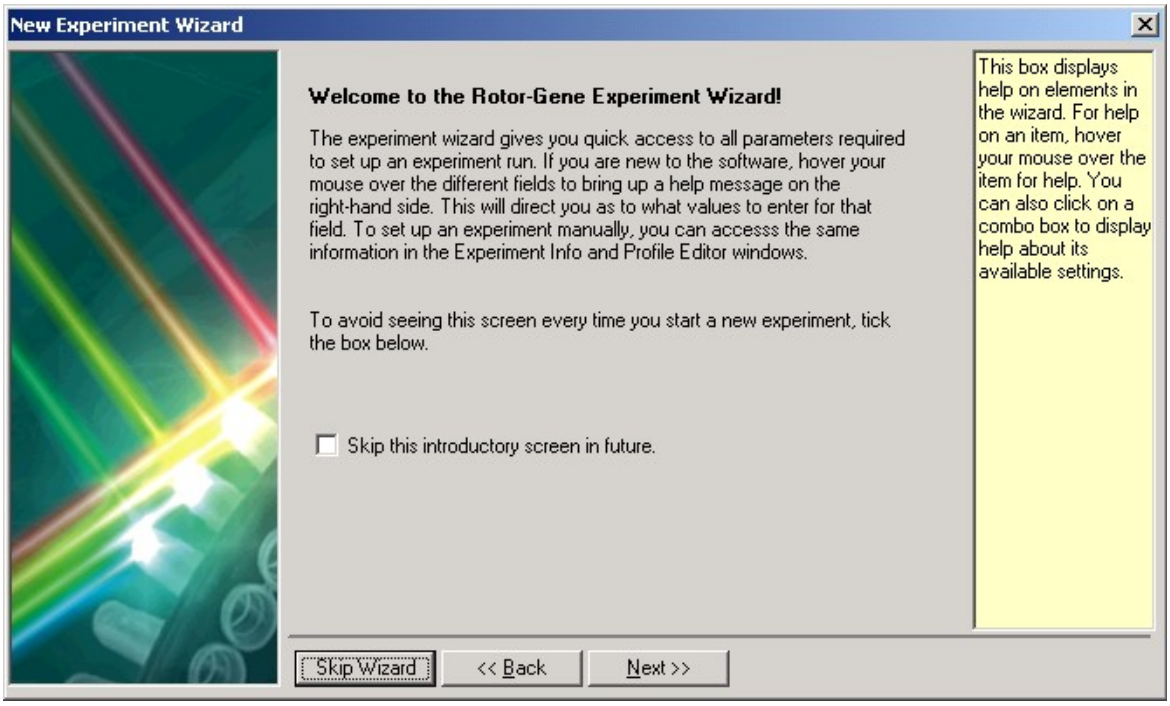
7 Quick Start Using The New Experiment Wizard

7.1 Welcome Screen

To perform a new experiment, click on the New icon .

By default the setup Wizard is active and you should see the wizard screen appear. If the Wizard does not appear tick the appropriate box in New Experiment.


New Experiment Wizard
Welcome to the Rotor-Gene Experiment Wizard!
The experiment wizard gives you quick access to all parameters required to set up an experiment run. If you are new to the software, hover your mouse over the different fields to bring up a help message on the right-hand side. This will direct you as to what values to enter for that field. To set up an experiment manually, you can accesss the same information in the Experiment Info and Profile Editor windows.
To avoid seeing this screen every time you start a new experiment, tick the box below.
Skip this introductory screen in future.
This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to display help about its available settings.
Skip Wizard
<< Back
Next >>

The wizard comes up with a welcome screen. After reading the introduction, the box **Skip this introductory screen in the future** can be ticked. The welcome screen will then automatically be skipped in the future.

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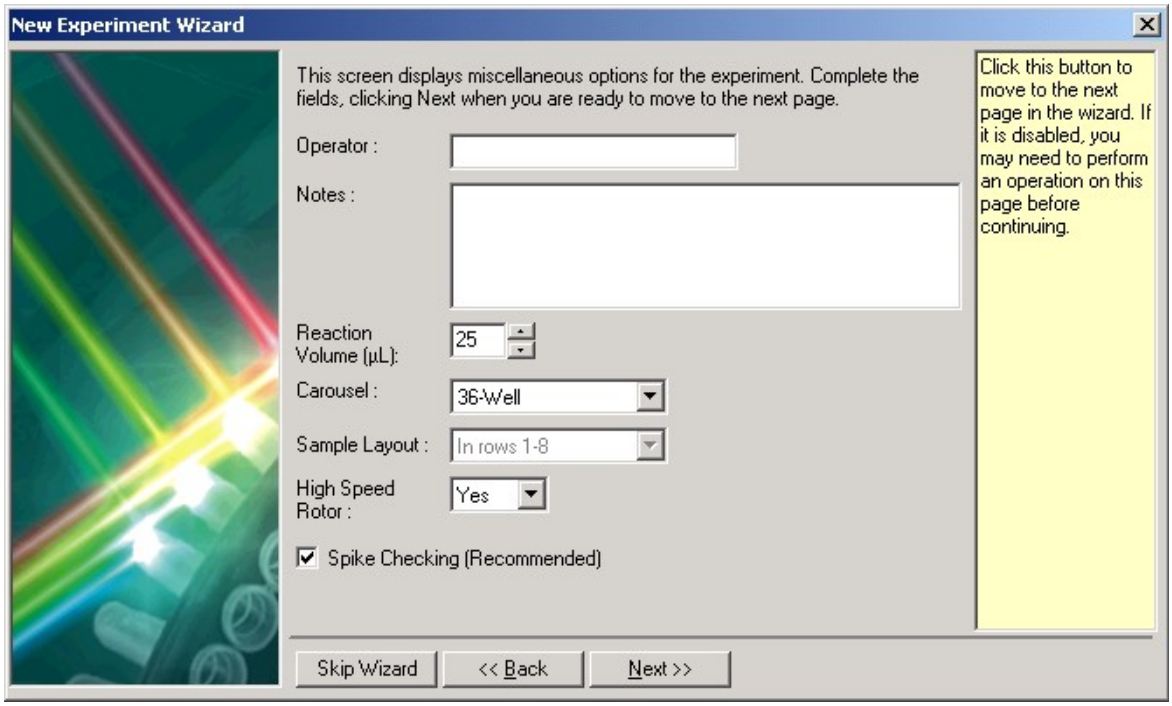
7.2 Page 1

The setup wizard guides you through all the necessary steps to setting up an experiment.


New Experiment Wizard
This screen displays miscellaneous options for the experiment. Complete the fields, clicking Next when you are ready to move to the next page.
Operator :
Notes :
Reaction Volume (µL):
25
Carousel :
36-Well
Sample Layout :
In rows 1-8
High Speed Rotor :
Yes
Spike Checking (Recommended)
Click this button to move to the next page in the wizard. If it is disabled, you may need to perform an operation on this page before continuing.
Skip Wizard
<< Back
Next >>

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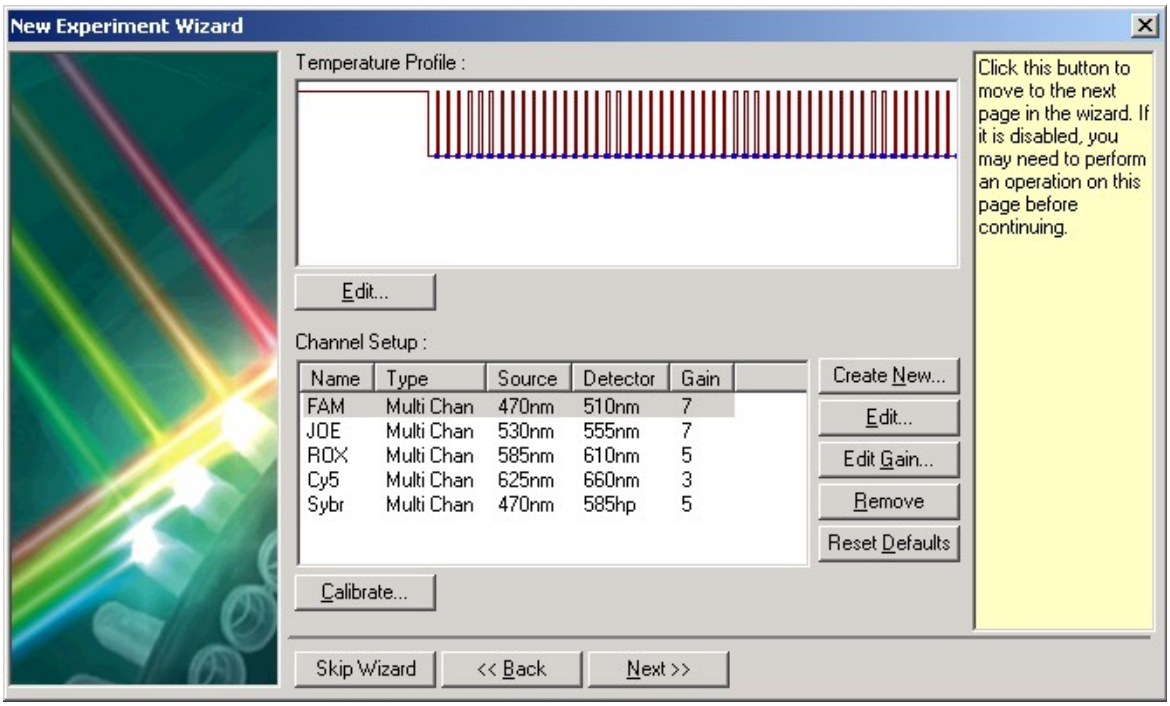
7.3 Page 2

In this screen the **Temperature Profile** and the **Channel Setup** can be modified. Simply press the appropriate buttons and modify as outlined in previous chapters.


New Experiment Wizard
Temperature Profile :
Edit...
Channel Setup :
Name Type Source Detector Gain
FAM Multi Chan 470nm 510nm 7
JOE Multi Chan 530nm 555nm 7
ROX Multi Chan 585nm 610nm 5
Cy5 Multi Chan 625nm 660nm 3
Sybr Multi Chan 470nm 585hp 5
Create New...
Edit...
Edit Gain...
Remove
Reset Defaults
Calibrate...
Click this button to move to the next page in the wizard. If it is disabled, you may need to perform an operation on this page before continuing.
Skip Wizard
<< Back
Next >>

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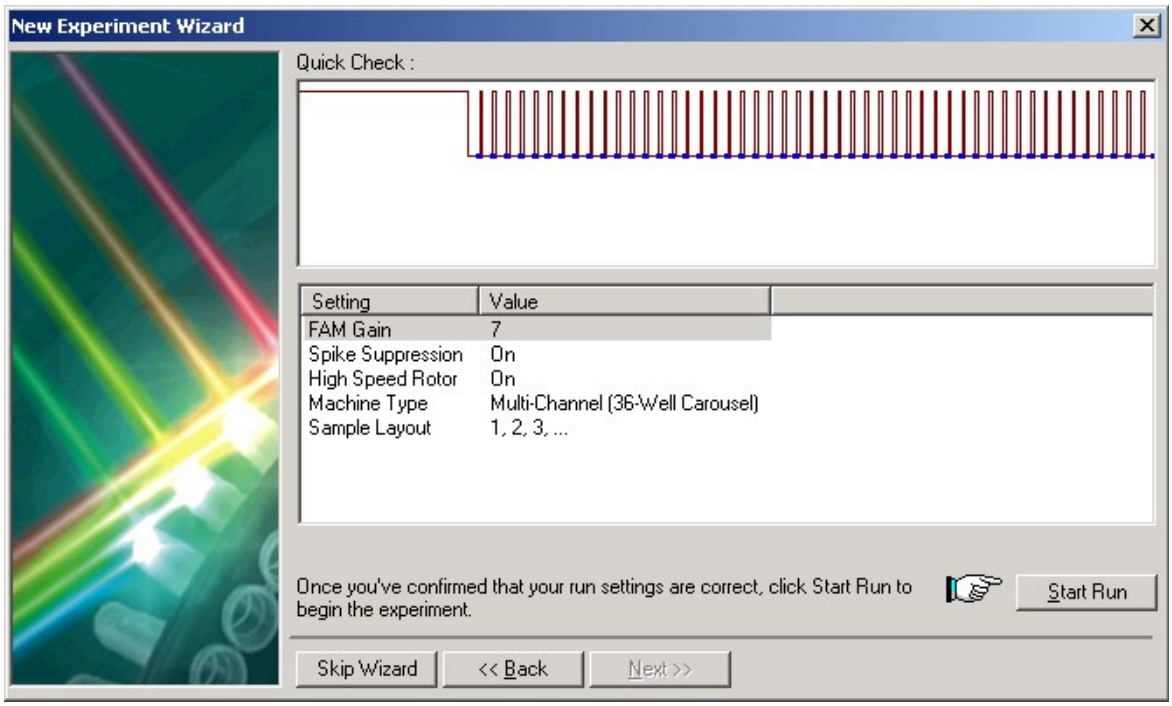
7.4 Page 3



The last screen gives a summary of what you are about to run. Always check the parameters. If you are satisfied with the parameters click **Start Run** and you will be prompted for a file name. Before starting the run make sure that the Rotor-Gene is in **READY** mode.

NOTE: If the unit does not display READY on the LCD screen, the detectors will not be activated and no data will be collected.

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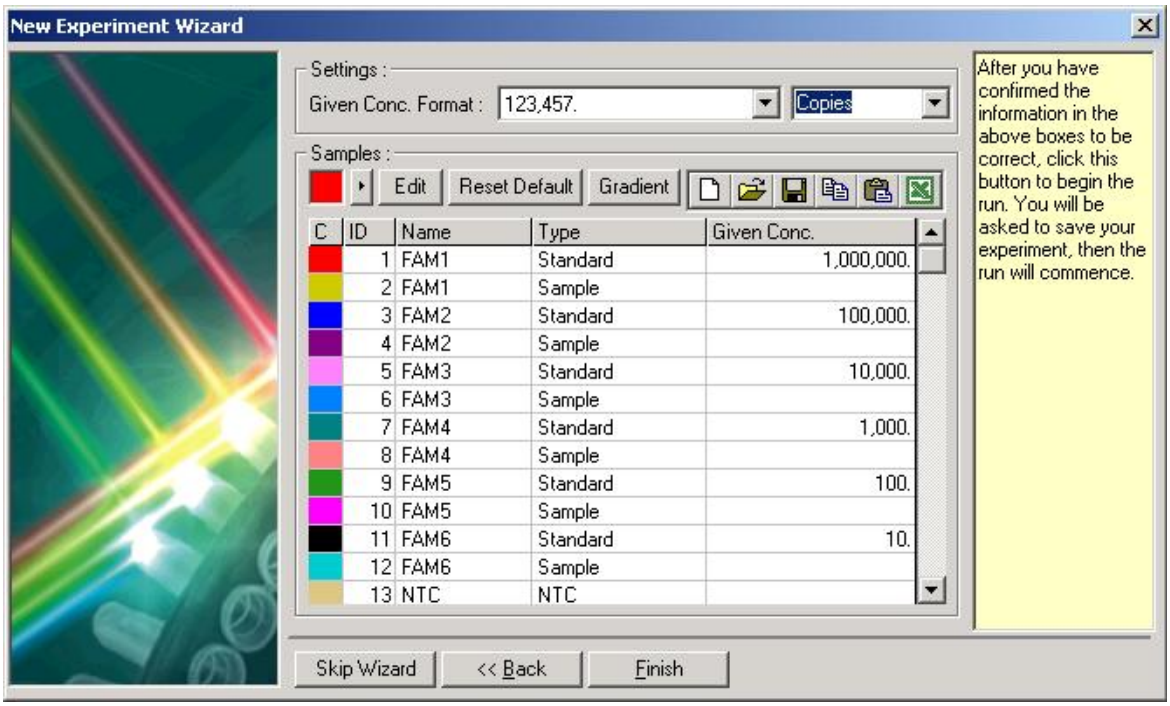
7.5 Page 4

Once the experiment has begun, you can enter in sample types and descriptions while you wait for it to complete. The functionality of this screen is identical to the Sample Editor.



8 Rotor-Gene Hardware Information

8.1 Filter Specifications

8.1.1 32 Well and Dual-Channel Machines

Filter specs:

Channel 1	Excitation:	470nm
	Detection:	510nm
Channel 2	Excitation:	530nm
	Detection:	555nm

Sybr-Green I is detected on Channel 1

Dual labeled probes can be detected on Channel 1 and Channel 2, when labeled with FAM or JOE (VIC), respectively.

Quenchers for the above fluorophores could be BHQ1 or TAMRA.

FRET style quenched probes could also be used labeled with FAM and JOE (VIC).

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NOTE: The above quenched dyes can be multiplexed in the same tube with no cross-talk in Channel 1 and less than 1% cross-talk in Channel 2.

8.1.2 Multi-Channel Machines

Sybr-Green I is detected on Channel 1 (FAM Channel) or Sybr Channel.

Dual Labeled Probes

Fluorophores	Excitation	Emission	Filter-Setup
5-FAM, 6-FAM:	495	520	FAM Channel
JOE	520	548	JOE Channel
TET	521	536	JOE Channel
HEX	535	556	JOE Channel
Cy3	550	570	470/585 hp or 530/585 hp
TAMRA	555	576	530/585 hp
Cy3.5	581	596	ROX Channel
ROX	575	602	ROX Channel
Red 640	625	640	Cy5 Channel
Cy5	649	670	Cy5 Channel

For multiplex reactions we recommend using FAM, JOE, ROX and Cy5, since those fluorophores after optimization give a cross talk less then 1%.

Quenchers to be used for the above dyes:

BHQ-1: 480 – 580
BHQ-2: 550 – 650
BHQ-3: 620 – 730
TAMRA for FAM or JOE (VIC)

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FRET Analysis

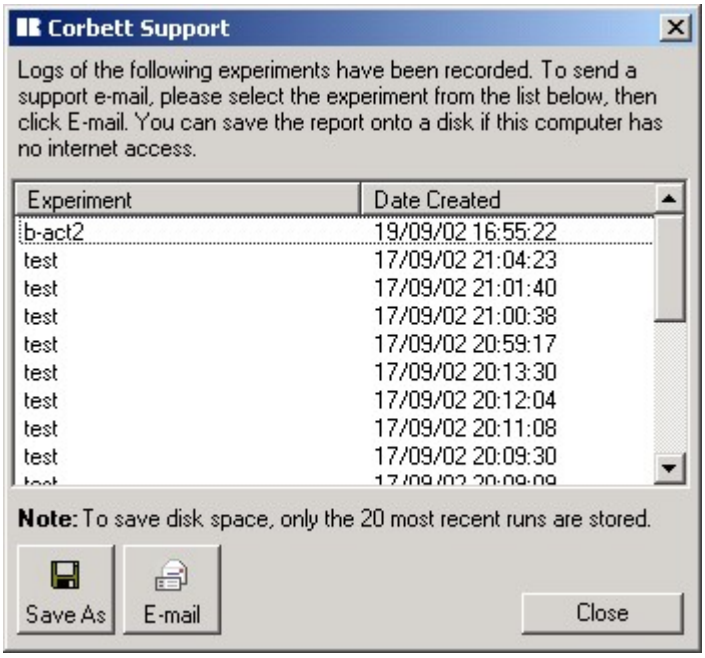
FAM and JOE for example have to be detected on the above mentioned channels (quenched FRET). If Cy5 has to be detected (normal FRET) the filter combination must be as follows:

FAM-Cy5, FAM-LC 640, FAM-LC 705	470/610 hp	FAM-BHQ1:	FAM Channel
JOE-Cy5	530/610 hp	JOE-BHQ1:	JOE-Channel

9 Troubleshooting

9.1 Log Archives

The software keeps an untouched copy of each experiment, along with diagnostic information in its Log Archive repository. By using the Help, Send Support Email option, you can send an email with all the diagnostic information the Corbett Research support technicians require:


Corbett Support
Logs of the following experiments have been recorded. To send a support e-mail, please select the experiment from the list below, then click E-mail. You can save the report onto a disk if this computer has no internet access.
Experiment
Date Created
b-act2 19/09/02 16:55:22
test 17/09/02 21:04:23
test 17/09/02 21:01:40
test 17/09/02 21:00:38
test 17/09/02 20:59:17
test 17/09/02 20:13:30
test 17/09/02 20:12:04
test 17/09/02 20:11:08
test 17/09/02 20:09:30
Note: To save disk space, only the 20 most recent runs are stored.
Save As
E-mail
Close

Previously, customers were required to send the experiment file, the temp.log and rot.log files. This is no longer necessary.

9.2 Troubleshooting Experiment Files

To troubleshoot experiments which did not occur as expected, enter the Profile Editor and ask the following questions:

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9.2.1 Initial Denature Step

Is the initial denature temperature and time for the Taq Polymerase used appropriate? Check the manufacture's description of the enzyme. The recommended temperature is 95°C and the times can vary from 2 min to 15 min, depending on the enzyme used. If genomic DNA has been used for the assay, the initial denature time should be at least 5 min depending on how the DNA has been extracted.

9.2.2 Cycling Profile

Have 50 ml volumes been run on the Rotor-Gene?
The Rotor-Gene is calibrated for 20 – 25 µl, in which case we recommend a denaturation time during cycling of 15 seconds. Some protocols specify 50 µl reactions in which case we recommend increasing the denaturation time during cycling to 30 seconds.

Is the denature temperature appropriate? Most double stranded DNA has melted at 95°C. In some cases temperatures from 90°C to 94°C may not denature the DNA properly. Due to partially denatured DNA, the accessibility of primers and probes could be reduced and could therefore reduce the reaction efficiency of the assay.

Is the denature time appropriate? 15 to 20 sec is usually enough to denature the amplicon, however, longer products might need up to 30 sec. A denature time of 60 sec is usually not required.

Is the annealing temperature and time appropriate? Check the Tm's of the primers and probes. The annealing time for Sybr-Green I is around 20 to 35 sec. Dual labeled probes are often run as a two step profile where annealing and extension steps are combined. In this case the annealing/extension step is between 45 and 60 sec and the temperature is usually 60°C. For FRET probes the annealing step is between 20 and 30 sec.

Is the extension temperature and time appropriate? Check the recommendations of the manufacturer's Taq Polymerase. For a product less than 300 bp the recommended time is between 15 and 30 sec.

At what step have data been acquired? For Sybr-Green I data should be acquired at 72°C. This is where most of the DNA is expected to be double stranded. As mentioned above, dual labeled probes are often run as two step assays. Data therefore should be acquired at the anneal/extension step. For FRET assays, data should be acquired at the annealing step. If in doubt about the appropriate data acquisition point, data could be acquired at several steps for comparison. If no raw data can been seen on the screen at all, go to edit profile and check if data was acquired at least at one point.

9.2.3 Melt Curve Analysis

Has a hold step at the same temperature as the beginning of the melt been inserted before starting the melt curve? If no hold step has been used before running a melt curve, the results in the melt curve analysis could be a steep increase in the first few cycles making the actual melt peak hardly visible.

For Sybr-Green I melt curves; was the melt curve run to 99°C? Some amplicons melt higher than 90°C. The expected melt peak might not occur, due to the fact that DNA has not melted.

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9.2.4 Gain Settings

Have the appropriate gain settings been chosen? In some cases it has been observed that curves in the raw data went off scale resulting in a straight line at a fluorescence of 100. Although quantitation of most data could still be performed, the gain should be reduced to a setting, where the raw data does not go off scale.
In some cases it has been observed that the fluorescence went off scale up from the first cycle. This is due to the fact that the gain setting was chosen too high. It might look like that no data has been acquired.
If a straight line at a fluorescence of 100 at the beginning of a melt curve is observed, the melt curve should be rerun using a lower gain. There is no need to rerun the amplification, Sybr-Green I or FRET samples can be reused to a certain extent.

Has the appropriate Quantitation Setting been chosen? In some cases it has been observed that the raw data show a perfect amplification but no data could be seen in the quantitation screen. Setting the Quantitation Setting to zero will show all data. The Quantitation Setting can be used to exclude small changes of fluorescence, due to probe degradations or other probe related effects, which do not show true amplification but rather a steadily increasing line.
If the quantitation curves can be excluded with a minimal Quantitation Setting the gain for the next run should be increased. Note: the gain setting has no influence on the actual data, the expected fold increase will be similar.

9.2.5 General Setup Conditions

Has the appropriate setup been chosen for the Rotor-Gene? To check, go to file menu then to setup and tick the appropriate boxes. If in doubt what each of the boxes means or if setup is not accessible, please contact the distributor.

Has the appropriate rotor for the run been chosen? Before running an experiment make sure that either the 36 or 72 well rotor has been chosen according to the rotor in the Rotor-Gene. Choosing the wrong rotor might either result in not acquiring every second sample (running 72 well rotor but 36 well setup was chosen) or acquiring double the amount of data that is actually in the rotor (running 36 rotor but 72 setup was chosen).

What version of the software is used on the Rotor-Gene? The software development for the Rotor-Gene is ongoing and freely downloadable from the Corbett Research web page (www.corbettresearch.com). Make sure that a recent version is uploaded and used.

9.2.6 Experiment Settings

If samples are not detected check also the channel setup. Make sure data has been acquired with the appropriate excitation and detection filters. This is particularly important when user defined filters have been setup.

Depending on the environment in which the Rotor-Gene is setup, spikes could be caused due to the noise from mains. The Rotor-Gene software has a build in spike suppression and will tell the user if spikes occurred during a run. If normal or medium spike suppression does not suppress spikes, high spike suppression should be ticked (version 4.4 or less). More recent versions have only one option to suppress spikes, by either enabling or disabling spike suppression. Spike suppression (high) is recommended and can be chosen in the experiment wizard.

What if all the above mentioned conditions were right, but the assay did not look as expected or did not work at all? In the restricted area of our homepage there is a file called "real-time

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summary". Steps are suggested on how to optimize Sybr-Green I, dual labeled probes and FRET reactions. In general, the expected fold increase of a Sybr-Green I reaction is between 5 and 50 times and a dual labeled reaction between 2 and 10 times.

9.3 Regional Settings In Windows 98

On some computers, the regional settings are configured in a contradictory manner which causes a conflict within the Rotor-Gene system. The problem occurs when different decimal settings are used for curencies and numbers. For example, if numbers are displayed in a "123 456,789" format, but currencies are shown as "$299,192.20", then there is a conflict, since the number "20,123" is ambiguous. On Windows 98 machines, this prevents the Rotor-Gene software from correctly interpreting numbers. This problem does not occur on Windows 2000.

To correct this setting, please follow the following steps. The names of these options may be different in your system's language.

- Click on the Start Menu.
- Click on Settings.
- Click on Control Panel.
- Double-Click on Regional Settings.
- Double-Click on Regional Options.
- Click on the Numbers tab.
- Note down which is the Decimal Symbol, and which is used as the Digit Grouping Symbol. On a German system, for example, the comma (,) will be used for Decimals, and the period (.) is used as a Digit Grouping Symbol.
- Click on the Currency tab.
- Change the Decimal Symbol and the Digit Grouping Symbol to be the same as on the Numbers tab.
- Click OK.
- Re-run the Rotor-Gene software.

10 Rotor-Gene 3000 Setup Instructions

To run software with the Rotor-Gene 3000 you have to follow these steps:

1. Setting up the Rotor-Gene 3000 Software for the first time:

Insert the Rotor-Gene 3000 CD into your CD drive.
Double Click on My Computer icon.
Double Click on Compact Disc Rotor-Gene 3000.
Double Click on RgSetup folder.
Double Click on executable file Rotor-Gene_X_Y_ZZ.exe (where X is Major version number, Y is Minor version number, ZZ is Revision version number).
Follow all Installer instructions.

2. Setting up the Rotor-Gene 3000 Software on a PC where it was already installed.

If you install Rotor-Gene 3000 Software on PC where it was installed before, it is necessary to uninstall any previous version of Rotor-Gene on your system.

In order to uninstall Rotor-Gene Software:
- Click on Start button on Taskbar,
- choose Settings, choose Control Panel, double click on Add/Remove Programs icon, choose Rotor-

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Gene
- Click Change/Remove
- Click Yes.

Then, to reinstall:
- Insert the Rotor-Gene 3000 CD into your CD drive.
- Double Click on My Computer icon.
- Double Click on Compact Disc Rotor-Gene 3000.
- Double Click on RgSetup folder.
- Double Click on executable file Rotor-Gene_X_Y_ZZ.exe (where X is Major version number, Y is Minor version number, ZZ is Revision version number).

11 Rotor-Gene 2000 Setup Instructions

To run software with Rotor-Gene you have to follow through these steps from 1-8 to install the board, the software drivers and the Rotor-Gene Software.

To run the software with the Rotor-Gene the Virtual Machine must not be ticked.

If you want to install a demonstration version of Rotor-Gene Software you have to do Step 7 only (Installing the Rotor-Gene Software). When you run software for demonstration purposes leave Virtual Machine ticked.

In order to change Virtual Machine box must click <File> <Setup> on the main Rotor-Gene Software screen.

If you Install Rotor-Gene Software on PC running Windows 95/98 you must disable Interrupt 3 in BIOS.

Boot the computer and Press Delete on keyboard simultaneously.
Choose INTEGRATED PERIPHERALS.
Change On Board Serial Port2: Disabled (Could be different on different PC i.e. Onboard Serial Port2 : 2F8/IRQ3)
Exit and Save Changes

If you install the Rotor-Gene Software on PC running Windows NT you must disable Interrupt 3 in BIOS.

Removing Com Port 2

Click on the Start Menu, Settings and Control Panel
Double-click on System. Then select the second tab Device Manager.
Click on the [+] sign next to Ports(COM+LPT) then click on COM2 If it still exist there.
Click on Properties.
Tick Disable in this hardware profile and click OK.
Click Close for Device Manager.
Reboot computer.

Disable hard disk power down

Click on the Start Menu, Settings and Control Panel
Double Click on Power Management
Choose Never for hard disks

Installing the Data Acquisition board

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Set the Interrupt jumper on the board to IRQ3.
Turn of the computer.
Plug in the board.
Turn on your computer again.

Installing the Computer Boards Software

Insert the Rotor-Gene 2000 CD into your CD drive.
Click on Start, Run.
Type in D:\RG2000\UNIVLIB5.04\DISK1\SETUP.EXE then press ENTER. If the drive letter of your CD Rom is one other than D, replace D with that drive letter.
The installation software will now start up. An screen will ask you to select which files to install. Select ONLY "Win 95/98/NT 32-bit Universal Library and Instacal", unticking any other options.
When the software asks you if it can make changes to AUTOEXEC.BAT, select "Yes". Do likewise for SYSTEM.INI.
The software will then ask you if it can reboot the computer. Select Yes.

Configuring The Computer boards Software

Click on Start, Run.
Type c:\cb\inscal32.exe and click OK.
Click on Install, Add Board…
Select the board CIO-DAS802 and click OK.
Double-click on the CIO-DAS802 board.
Set the Interrupt Level to 3.
Set Wait State Disabled.
For each Channel from 0 to 7 in channel configuration, change from DIFF to SE.
Click OK.
Select File, Exit.

Installing the Rotor-Gene Software

If you install Rotor-Gene Software first time go to 7.a .
Before installing any new version of the software, it is necessary to uninstall any previous version of Rotor-Gene on your system.
Click on Start, Settings, Control Panel.Double click on Add-remove software.
In the list box at the bottom of the window, for each copy of Rotor-Gene in the list, click on Rotor-Gene, then click on Add/Remove. Click on Close.
You can now run setup.exe, located in D:\ RGSetup\Setup.exe
If the drive letter of your CD Rom is one other than D, replace D with that drive letter.

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12 Rotor-Gene 2000 IMPORTANT SETTINGS

IMPORTANT SETTINGS THAT SHOULD NOT BE CHANGED ON THE PC

1) DO NOT Turn off hard disks. Ensure Power-down on hard disks is set to NEVER.

2) DO NOT Put the system on Standby. Ensure Enter Standby mode is set to NEVER.

3) DO NOT Install any peripheral devices that are using Interrupt 3.

4) DO NOT Install computer on network if the network card is using Interrupt 3.

5) DO NOT Enable Interrupt 3 in BIOS.

6) DO NOT install custom screen savers.

NB: These notes only apply to the Rotor-Gene 2000.

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